Evaluation of Grapevine Rootstocks for Use Against Grapevine Fanleaf Virus

By

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ABSTRACT

One of the most destructive grapevine viruses is grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration. This virus is vectored from root-to-root by the dagger nematode (*Xiphinema index*) and can result in crop losses of up to 80% by greatly reducing fruit set and causing formation of 'shot berries,' small, seedless berries that do not mature. Currently, fanleaf degeneration is controlled by grafting vines onto the rootstock O39-16, which suppresses the expression of fanleaf degeneration symptoms in the scion. However, the *Vitis vinifera* parentage of O39-16 raises concerns about the rootstock's long-term susceptibility to grape phylloxera and other pests and diseases. Additionally, O39-16 is susceptible to root-knot nematodes and induces high vigor to scions grafted on it. Due to these reasons, breeding efforts to produce alternative fanleaf degeneration rootstocks have continued.

In 2007, 101-14 Mgt. was crossed with *Muscadinia rotundifolia* cv. Trayshed and the resulting progeny have been growing at the University of California, Davis. *Muscadinia rotundifolia* is the source of rootstock-induced tolerance observed in O39-16, and 101-14 Mgt. (*V. riparia* x *V. rupestris*) is a popular commercial rootstock commonly chosen for its ease of propagation, moderate nematode and phylloxera resistance, and the ability to control vigor to scions grafted on it. We quantified GFLV resistance and fanleaf degeneration tolerance in the progeny from this cross and studied the inheritance of these traits. Both traits segregated in the 101-14 x Trayshed population as quantitative traits controlled by multiple genes.

Additionally, we developed a novel method that utilized digital imaging to obtain fruit set ratios for individual grape clusters as a means to quantify fanleaf tolerance in vines. Utilizing this method, we identified six progeny from this hybrid population (07107-012, 07107-043, 07107-091, 07107-112, 07107-135, and 07107-148) that were able to control fanleaf degeneration as well as O39-16 and exhibited desirable viticultural traits. This study is the first to have extensively quantified fruit set as a method to determine the degree of fanleaf degeneration tolerance induced by a rootstock to GFLV-infected vines.

In addition to exploring the 101-14 x Trayshed breeding population, we also studied five recently-released rootstocks with broad and durable nematode resistance. Although these five rootstocks (GRN-1, GRN-2, GRN-3, GRN-4, and GRN-5) possess resistance to *X. index*, their ability to induce fanleaf tolerance (such as the case in O39-16) is unknown and the overall performance of these rootstocks on a GFLV-infested site has not been thoroughly evaluated. This manuscript presents the first evaluation of these new rootstocks on a fanleaf site in the San Joaquin Valley in California. After ten years of growth, all five GRN rootstocks performed comparably to O39-16. The low GFLV infection rate in vines grafted on GRN-1 shows the potential for GRN-1 to become a viable alternative to O39-16. Although further studies are needed to evaluate the long-term performance of these rootstocks, these initial results are promising.

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Grapevine fanleaf virus resistance in a 101-14 Mgt. x *Muscadinia rotundifolia* breeding population and the *M. rotundifolia*-based rootstock GRN-1

ABSTRACT

Grapevine fanleaf virus (GFLV) causes fanleaf degeneration, one of the most economically severe viral diseases affecting grapevines worldwide. The disease can result in crop losses of up to 80% by greatly reducing fruit set, leading to small, seedless 'shot berries.' *Muscadinia rotundifolia*, a North American grape species, has been identified as a valuable source of GFLV resistance. The objective of this work was to quantify GFLV resistance in the progeny from a cross between the susceptible commercial rootstock 101-14 Mgt. and *M. rotundifolia* cv. Trayshed, and to study the inheritance of this trait. GFLV resistance in the commercial rootstock GRN-1 (*Vitis rupestris* x *M. rotundifolia*) was also studied due to its *M. rotundifolia* parentage. Plants were bench-graft inoculated using hardwood cuttings of GFLV-infected *V. vinifera* cv. Cabernet Sauvignon. Fifty individuals of the 101-14 x Trayshed population and hardwood cuttings of GRN-1 were inoculated. Six months after grafting and growing in a greenhouse, actively growing roots from the plants were assayed for GFLV using RT-qPCR. GFLV resistance segregated in the 101-14 x Trayshed population as a quantitative trait controlled by multiple genes. All rootstocks in the study had statistically similar or lower levels of GFLV resistance as compared to O39-16, the tolerant control. GRN-1 performed relatively poorly in the study, showing some degree of resistance, but not nearly as high as other screened genotypes. Our results provide insight

into the inheritance of GFLV resistance from *M. rotundifolia* and continue our progress toward developing new rootstocks to ameliorate the effects of fanleaf degeneration.

INTRODUCTION

Grapevines (*Vitis* spp.) are one of the world's most widely grown crops, with recent worldwide production estimated at 7.6 million hectares of grapevines and 74 million metric tons of grapes (Reynolds, 2017). However, grapevines are susceptible to 70 reported viruses that can cause major economic damage (Martelli, 2014). One of the most destructive grapevine viruses is grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration. The disease can result in crop losses of up to 80% by greatly reducing fruit set and causing formation of 'shot berries,' small, seedless berries that do not mature (Andret-Link et al., 2004). Leaf symptoms include an unusually wide open petiolar sinus and exaggerated teeth on the leaf margins which gives the leaf a "fan-shape," yellow mosaic on the leaf blades, and yellow vein banding along the main veins of mature leaves. Vine shoots can also be deformed, showing zigzag growth, short internodes, and irregular branching (Andret-Link et al., 2004).

The virus spreads across vineyards through its vector, the dagger nematode *Xiphinema index*. The usage of nematicides to combat this soil-borne pest is restricted due to the high toxicity and damage to the environment caused by the chemicals (Van Zyl et al., 2012, Villate et al., 2012). Several nematicides have been banned in Europe in the past thirty years (Bouquet et al., 2000). Crop rotation and fallow periods are too costly due to the high premium placed on vineyard land and the long fallow period required (six to ten years) for the elimination of *X. index* (Demangeat et al., 2005). Therefore, breeding efforts have

focused on combating the disease by developing a rootstock that would be resistant to the virus when probed and fed on by *X. index*. After 10 years of field screening, the rootstock O39-16 was released in 1991 to control fanleaf degeneration in vineyards known to harbor *X. index* (Walker et al., 1991). This rootstock is a *V. vinifera* cv. Almeria x *M. rotundifolia* cv. 'Male No. 1' hybrid. Interestingly, despite its resistance to *X. index*, the virus is still successfully vectored into the roots by *X. index*'s deep probing and virus levels in the scion after seven years are similar to those grafted on susceptible rootstocks (Walker et al., 1994b). However, foliar symptoms are reduced and the disruption of fruit set usually accompanied by viral infection is not observed in scions grafted on O39-16 (Walker et al., 1994b).

Although O39-16 suppresses the expression of fanleaf degeneration symptoms in the scion, it does not alter GFLV infection. It is unclear to what degree the virus multiplies in the rootstock and whether this is correlated to the tolerance conferred to a scion, or if other mechanisms are at play. Resistance is the plant's ability to suppress virus multiplication to a degree (either completely or partially), and tolerance is the ability to lessen the damage caused by virus infection (Oliver & Fuchs, 2011). This differentiation between resistance and tolerance is critical because the terms describe different virus-host interactions that impact disease management in distinct ways. In the case of O39-16, although the rootstock is resistant to the virus and to the nematode's feeding it does not prevent its movement into the scion where it multiplies normally. However, the fanleaf degeneration symptoms of poor set are prevented, seemingly by some compound generated by the O39-16 rootstock. In this study, a new breeding population was examined with the goal of identifying additional new rootstocks capable of inducing fanleaf tolerance and possessing higher levels of GFLV resistance than O39-16.

Although O39-16 has been effective in the control of fanleaf degeneration, there are three key reasons to breed new rootstocks with resistance or tolerance to fanleaf degeneration. The *V. vinifera* parentage of O39-16 raises concerns about the rootstock's long-term susceptibility to grape phylloxera and other pests and diseases (Walker et al., 1994a). Phylloxera devastated the *V. vinifera*-based European vineyards 150 years ago and it also led to the failure of the grape rootstock AXR-1 (a rootstock with 50% *V. vinifera* parentage) in California 35 years ago (Granett et al., 1996). Notably, O43-43, a sibling of O39-16, also shares in the ability to induce fanleaf tolerance to scions, but it was promptly removed from distribution after collapsing to phylloxera (Walker et al., 2014). Although O39-16 has remained resistant to phylloxera, breeding for fanleaf resistance should continue given the history of phylloxera susceptibility in *V. vinifera* containing rootstocks. Secondly, O39-16 induces high vigor to scions grafted on it (Walker et al., 1994b). High vigor can result in poor bud fruitfulness and low quality fruit in addition to requiring more labor to attain balanced vine canopies (Kliewer & Dokoozlian, 2005). Lastly, O39-16 is susceptible to root-knot nematodes (Walker et al., 1994b). Like other plant-parasitic nematodes, root-knot nematodes feed on roots and eventually cause necrosis of active roots, reducing water and nutrient uptake (Nicol et al., 1999). At the moment, usage of O39-16 is restricted to fanleaf degeneration sites due to these less than ideal viticultural characteristics.

To address these concerns, crosses of 101-14 Mgt. x *M. rotundifolia* cv. Trayshed were made several years ago and the resulting progeny have been growing at the University of California, Davis. *Muscadinia rotundifolia* is the source of rootstock-induced fanleaf degeneration tolerance observed in O39-16, but pure *M. rotundifolia* is known to be difficult to root from dormant cuttings and generally forms incompatible unions when grafted to *V.*

vinifera cultivars (Bouquet, 1980, Walker & Jin, 1998). 101-14 Mgt. (*V. riparia* x *V. rupestris*) is a commercially used rootstock that is popular due to its ease of propagation, good phylloxera and nematode resistance and ability to control vigor in scions grafted to it (Bettiga et al., 2003). Since this cross does not contain any *V. vinifera* parentage, it could generate new rootstock alternatives without risk of phylloxera susceptibility. GRN-1 (*V. rupestris* A. de Serres x *M. rotundifolia* cv. Cowart) is a rootstock released in 2008 with strong and broad nematode resistance (Walker et al., 2014). Its *M. rotundifolia* background along with its lack of any *V. vinifera* parentage also makes it an ideal candidate for this study to identify new rootstocks with GFLV resistance.

The goal of this study was to quantify GFLV resistance for selected individuals in the 101-14 x *M. rotundifolia* cv. Trayshed population as well as in the *M. rotundifolia*-based rootstock, GRN-1. GFLV resistance in these rootstocks was compared to resistance in rootstocks known to confer tolerance to fanleaf degeneration (O39-16 and O43-43) and rootstocks known to confer no tolerance to fanleaf degeneration (St. George and 101-14). This study presents findings on the inheritance of GFLV resistance and identifies three individuals (07107-065, 07107-002, and 07107-133) that show promise as potential rootstocks capable of controlling GFLV due to their ability to significantly suppress viral replication. This study also provides necessary phenotypic data that lays the foundation for future research directed at breeding virus tolerant rootstocks.

MATERIALS AND METHODS

Plant Material

All GFLV-infected scion material (*V. vinifera* cv. Cabernet Sauvignon) was collected from a vineyard in Rutherford, CA. Its sole infection with GFLV was verified by RT-qPCR by Foundation Plant Services (FPS).

Rootstock material for this study was obtained from a research vineyard at the University of California, Davis. Crosses of 101-14 Mgt. x *M. rotundifolia* cv. Trayshed were made in 2007 and the resulting progeny were grown in a vineyard free of *X. index*. This population will hereby be referred to as the 07107 population. There are 85 surviving individuals in this population and 50 of those genotypes were selected for the study. The other 35 individuals in this population were too weak to provide adequate dormant cuttings for the project, so they were excluded from the available pool of plants. Cuttings of certified GRN-1 (*V. rupestris* x *M. rotundifolia*) were provided by FPS and verified to be virus-free.

The tolerant control rootstocks (O39-16 and O43-43) and the susceptible control rootstocks (St. George and 101-14) were FPS certified and verified to be virus-free. Virus-free *V. vinifera* cv. Almeria (the *V. vinifera* parent of O39-16 and O43-43) and virus-free *V. vinifera* cv. Cabernet Sauvignon were also obtained from FPS to act as additional rootstock controls for the study.

Grafting/Inoculation

In January 2018, dormant cuttings (about 0.5-1 cm in diameter) were taken for bench grafting and stored at 4^{o}C until used. The scion cutting was selected to be about 10 cm in length with one dormant bud. The rootstock cutting was 18-22 cm in length with all nodal

buds removed. The base of the scion was grafted to the rootstock with an omega grafting machine. Paraffin wax was used to seal the graft union and the exposed top surface of the scion. Fifteen bench grafts were made for each rootstock. All rootstocks were grafted with the GFLV-infected scion material described above with the purpose of inoculating GFLV into the rootstocks. The tolerant control rootstocks (O39-16 and O43-43) and the susceptible control rootstocks (St. George and 101-14) were also grafted with virus-free Cabernet Sauvignon scions to act as virus-free controls. Bench grafts were callused at 28^{o}C in a moistened 1:1 mix of perlite and vermiculite for four weeks and then transferred into cardboard sleeves with a moistened 1:1 mix of perlite and vermiculite and placed in a fog room at 28^{o}C for two weeks to induce root formation.

Plant Growth

Three replicates of each graft combination that had properly rooted and callused were transferred to individual pots and grown in a greenhouse. Plants were grown in tall rectangular pots (7 cm x 7 cm x 20.3 cm) filled with equal parts perlite, vermiculite, and peat (1:1:1). Temperature in the greenhouse was set to 30^{o}C/20^{o}C day/night and the photoperiod was maintained at 16 hours with supplemental lighting. Plants were watered every two days with a modified Hoagland's solution.

Relative Virus Quantification

Six months after being transferred to the greenhouse, the roots of each plant were assayed for GFLV using RT-qPCR. Approximately 1 g of living root tissue was collected from each plant, temporarily placed on ice, and then transferred to a freezer at -80^{o}C until

processed. RNA isolation was performed using a CTAB method as described by Blanco-Ulate *et al.* (2013) and the RNA pellet was further purified using the *Quick*-RNA Miniprep Kit (Zymo Research). RNA concentration and purity were measured using a Nanodrop 2000c Spectrophotometer (Thermo Scientific, Inc.). cDNA was synthesized from 1 µg of prepared RNA using qScript™ XLT cDNA SuperMix (QuantaBio) in a volume of 20 µL. The resulting cDNA was diluted with an additional 80 µL of double distilled water. RT-qPCR was performed on a StepOnePlus PCR System using Fast SYBR Green Master Mix (Applied Biosystems). All reactions were performed with the following cycling conditions: 95°C for 10 minutes, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. 18S rRNA was used as an internal control. Primer sequences used for RT-qPCR were as follows: GFLV-F: GTTGTGTGTTAGGTATGGGAGGTACTATTA; GFLV-R: TTCCACATACACCCCGGGATA; 18S rRNA-F: GTGACGGAGAATTAGGGTTCGA; 18S rRNA-R: CTGCCTTCCTTGGATGTGGTA (Osman et al., 2008). Positive controls as well as no template (negative) controls were included. Virus amounts were evaluated by the comparative C_T method (Schmittgen & Livak, 2008).

A subset of plants was randomly chosen for scion shoot tip sampling to verify consistent infection across the plants. Ten progeny were randomly selected from the 07107 population for this confirmation as well as GRN-1, O39-16, 101-14, St. George, and *V. vinifera* cv. Cabernet Sauvignon. Virus status was evaluated on actively growing shoot tips with the previously described methods. Shoot tips were collected at the same time as root tissue and all three replicates of each selected graft combination were sampled.

Statistical Analysis

A completely randomized design was used for this study. Analysis of variance (ANOVA) was performed in RStudio v1.3.1056 running R v4.0.2 with the *car* and *DescTools* software packages (Fox & Weisberg, 2018, Signorell et al., 2020). The Anderson-Darling test, Levene's test, and outlier test were used, respectively, to test for assumptions of normality, equality of variances, and outliers. $2^{-\Delta C_T}$ values were square-root transformed to meet assumptions of normality and equality of variances. Tukey's test was performed for pairwise means comparisons. A Pearson's correlation test was performed to determine if there was a correlation between GFLV levels in the scions to the rootstock concentrations in the subset of plants randomly chosen for shoot tip sampling.

RESULTS

Graft Success Rate

From the 50 rootstock genotypes selected from the 07107 population for this study, 36 genotypes survived with three or more biological replicates from the original fifteen bench grafts. Thirteen of these 36 genotypes had a survival rate of 90% (Table 1.1). The 14 rootstock genotypes with less than three surviving biological replicates were discarded from the study. GRN-1 and all controls had a 100% survival rate except for O43-43 (93%). Genotypes 07107-046, 07107-062, and 07107-065 had only moderate grafting success but were easy to root. Notable genotypes from the 07107 population that both grafted and rooted well after callusing were: 07107-007, 07107-018, 07107-091, 07107-112, and 07107-135.

Virus Quantification

Significant differences among rootstock genotypes were observed ($P < 0.001$). To interpret differences among genotypes, Tukey's test was performed (Table 1.2). O39-16 harbored the second lowest level of GFLV six months post-grafting to a GFLV-infected *V. vinifera* cv. Cabernet Sauvignon scion. 07107-065 harbored the lowest level of GFLV. This genotype, as well as 07107-002, O43-43, and 07107-133, had virus levels that were statistically similar to O39-16 (Table 1.2). No rootstock in the study harbored statistically lower levels of GFLV compared to the level observed in O39-16. St. George, a rootstock that confers no tolerance to fanleaf degeneration as well as being a rootstock that contains no *M. rotundifolia* background, harbored the highest level of GFLV in this study. Two genotypes from the 07107 population, 07107-110 and 07107-070, harbored statistically similar levels of GFLV as St. George. Almeria, the *V. vinifera* parent of O39-16, was also in this grouping of most susceptible rootstocks (Table 1.2). GRN-1, a *V. rupestris* x *M. rotundifolia* hybrid, was intermediate in its performance. 101-14 performed very poorly in the study with only two other rootstocks in the study (07107-070 and St. George) harboring a statistically higher level of GFLV than 101-14 (Table 1.2). No GFLV was detected in the virus-free controls.

Plotting the mean virus levels for each genotype shows that GFLV resistance did segregate in the 07107 population, but the trait appears to be quantitatively controlled because of the wide range and distribution of resistance levels in the population (Figure 1.1).

GFLV Detection in Scions

GFLV was detected in all scions tested, showing that virus infection persisted in the scion after grafting (Figure 1.2). A Pearson's correlation test between GFLV levels in these

selected scions and their respective rootstock resulted in a *p*-value of 0.9998, meaning there was no correlation between the GFLV levels in the scions to the rootstock concentrations.

DISCUSSION

Although O39-16 has been very effective to date at controlling fanleaf degeneration, the *V. vinifera* background casts doubt on the rootstock's long-term resilience against grape phylloxera and the rootstock is already known to be susceptible to root-knot nematodes (Walker et al., 1994a). There have been few studies regarding GFLV resistance and fanleaf degeneration tolerance, with most of the current studies incorporating GFLV resistance through genetic engineering (Hemmer et al., 2018, Pakbaz et al., 2018). Although it is known that O39-16 can confer tolerance to fanleaf degeneration, there have been no comprehensive studies regarding its ability to suppress GFLV replication in the rootstock. The level of virus resistance in the rootstock may be critical information related to a rootstock's ability to confer disease tolerance to scions because the higher viral load in the rootstock may weaken the vine and/or cause higher virus titer in the scion. The present study quantified GFLV resistance in O39-16, its sibling O43-43, one of their parents (*V. vinifera* cv. Almeria), and a promising breeding population composed of progeny from a cross of the easily rooted 101-14 Mgt. rootstock and pest and disease resistant, but rootable *M. rotundifolia* cv. Trayshed.

Vitis vinifera cv. Almeria is the maternal parent of both O39-16 and O43-43. Almeria showed relatively high levels of GFLV in this study (Figure 1.1). Thus, we confirm that the rootstocks' paternal parent, *M. rotundifolia* cv. 'Male No. 1,' is the genetic source of GFLV resistance in O39-16 and O43-43. No pure *M. rotundifolia* cultivars were included in this

study due to the difficulty in grafting *M. rotundifolia* to *Vitis* spp. scions and the inability of *M. rotundifolia* to root from dormant cuttings (Bouquet, 1980).

O39-16 and O43-43 are the only known rootstocks that confer tolerance to fanleaf degeneration to scions (Walker et al., 1991). When comparing GFLV resistance in these tolerant rootstocks with other rootstocks used in industry, it is clear that the tolerant rootstocks carry some degree of GFLV resistance since both O39-16 and O43-43 suppressed replication of GFLV compared to St. George and 101-14 (Table 1.2 and Figure 1.1). This may indicate a possible correlation between a rootstock's ability to induce fanleaf tolerance and its ability to suppress GFLV replication, which will be explored in Chapter 2. However, although O39-16 and O43-43 significantly suppressed viral replication, the two rootstocks still had trace, but detectable, amounts of GFLV when compared to the negative controls, showing that they are not completely resistant.

It is expected that O39-16 and O43-43 are not completely resistant to GFLV because the rootstocks still allow for vectoring of the virus when probed by *X. index*. Although O39-16 and O43-43 are resistant to feeding damage by *X. index*, the nematode can still probe on the roots and vector GFLV into the plant (Walker et al., 1991). Although the results of this study indicate that these rootstocks do suppress viral replication in the rootstock, this trait does not change the inevitable infection of the scion as the virus travels up through the rootstock to arrive in the scion where it replicates normally. Scions grafted on these tolerant rootstocks show similar levels of GFLV compared to scions grafted on other rootstocks (Walker et al., 1994b). However, foliar symptoms are reduced and the disruption of fruit set usually accompanied by viral infection is not observed (Walker et al., 1994b). The induced disease tolerance provided by these rootstocks will be further discussed in Chapter 2.

These results also show that it is possible for O39-16 to act as a reservoir for GFLV in vineyards. It may be that *X. index* probing an infected O39-16 rootstock are still able to pick up the virus and vector it to other vines, which needs to be explored in a future study. This is especially concerning if there are vines in adjacent blocks that are grafted on susceptible rootstocks. However, even though *X. index* can vector GFLV, the nematode cannot successfully feed and reproduce on O39-16 (McKenry et al., 2001, Ferris et al., 2013). Because of this, there is potential that O39-16 can act as a natural nematicide and that when a O39-16 vineyard is pulled after a generation the nematode may no longer exist. There are many issues that may confound this process, including the life-span of nematodes, the lateral movement of roots from adjacent vineyards on susceptible rootstocks, and the impact of the soil environment on *X. index* survival. Further studies are needed on the use of O39-16 as a method to eradicate *X. index* (and subsequently GFLV) from infested fields.

101-14 Mgt., the maternal parent of the 07107 population, showed very little ability to suppress viral replication when compared to its offspring (Table 1.2 and Figure 1.1). This validates that GFLV resistance in the 07107 population is most likely inherited from the *M. rotundifolia* cv. Trayshed parent. This *M. rotundifolia* cultivar was unable to be included in the present study due to its inability to root from dormant cuttings and its graft incompatibility with *Vitis* spp. scions. However, previous studies conducted with other methods (e.g. green grafting) have observed GFLV resistance in *M. rotundifolia*, so we conclude that Trayshed does have at least some degree of GFLV resistance (Bouquet, 1981, Walker et al., 1985).

Muscadinia rotundifolia is known to be difficult to root from dormant cuttings and it generally forms incompatible unions when grafted to *V. vinifera* cultivars (Bouquet, 1980).

Therefore, when considering the 07107 population (101-14 x *M. rotundifolia* cv. Trayshed) as a rootstock population, it is important to be aware of the level of graft success observed in a population of seedlings. Of the 50 individuals chosen for this study, 36 individuals had the required graft success rate of at least 20% (to produce the three biological replicates needed). However, for industry standards, a success rate of 80% or more is highly preferred. Considering this, only thirteen of the genotypes tested in this study met this standard. As expected, all controls in this study met this 80% minimum success rate since they are standard commercial rootstocks currently used or once used in viticulture (GRN-1, O39-16, O43-43, St. George, and 101-14) or *V. vinifera* controls (Almeria and Cabernet Sauvignon).

As previously mentioned, all unions were callused for four weeks post bench-grafting rather than the normal two weeks, to improve grafting success, especially for GRN-1, O39-16, and O43-43 which have been regarded as being difficult to propagate (Walker et al., 2014). Further studies should be conducted regarding the propagation of *M. rotundifolia*-based rootstocks to maximize graft success rate while maintaining nursery production efficiency. Variations in traditional propagation techniques (such as prolonging callusing time or time in the fog room or collecting wood earlier or later than usual) should be explored. The *M. rotundifolia* background of these rootstocks may require special treatment for maximum propagation success, but the trade-off of broad disease and pest resistance may be worth the additional time and labor (Wiedeman-Merdinoglu et al., 2002, Ruel & Walker, 2006, Louime et al., 2011).

The 07107 population segregated for GFLV resistance (Figure 1.1). Due to the lack of any distinctive segregation ratio and the apparent wide gradient of resistance levels, we conclude that GFLV resistance is a quantitative trait controlled by multiple genes. The

phenotypic data obtained in this study provides a foundation for a possible future study to locate a genetic marker for this trait. However, a much larger population size will be required. There are polymorphic markers available for *M. rotundifolia* and an SSR marker-based genetic map of *M. rotundifolia* has been previously published (Riaz et al., 2012).

Of the 36 genotypes from the 07107 population that were tested in this study, three of these genotypes had levels of GFLV resistance that were statistically the same as O39-16 (Table 1.2). Although there were no rootstocks that completely resisted GFLV, these three genotypes (07107-065, 07107-002, and 07107-133) show promise as potential rootstocks that can control GFLV due to their ability to significantly suppress viral replication. The possibility of these three rootstocks to induce fanleaf degeneration tolerance to scions will be discussed in Chapter 2. 07107-133 is especially promising due to its graft success rate exceeding 90% in this study (Table 1.1).

In addition to the three genotypes listed above, there were eleven other genotypes that showed statistically similar levels of GFLV resistance as O43-43, another rootstock known to confer fanleaf tolerance to scions (Table 1.2). Special attention should also be given to these eleven additional genotypes in later studies. Although these eleven genotypes harbored statistically higher levels of GFLV than O39-16, their statistical similarity to O43-43 may indicate potential to confer fanleaf tolerance to scions. Notably, six of these eleven genotypes (07107-007, 07107-043, 07107-077, 07107-091, 07107-112, 07107-135) showed promising graft success rates exceeding 90% in this study.

GRN-1 (*V. rupestris* cv. A. de Serres x *M. rotundifolia* cv. Cowart) was shown to have moderate GFLV resistance when compared to the other rootstocks in this study. Pairwise comparisons conducted through the Tukey's test showed that GRN-1 showed

significantly higher GFLV levels than O39-16 and O43-43, but still significantly lower than 101-14 and St. George (Table 1.2). When considering that GRN-1 has *M. rotundifolia* background, it is unsurprising that the rootstock shows some degree of GFLV resistance. It is unclear whether this moderate GFLV resistance will translate into the ability to confer fanleaf tolerance to scions. As of the writing of this manuscript, long-term field studies are underway to study the performance of GRN-1 at fanleaf sites.

GFLV was detected in all scions selected for infection verification (Figure 1.2). We observed consistent infection across all tested scions, giving greater confidence to the accuracy of the study. This likely indicates that any observation of low GFLV levels in a rootstock genotype was due to the rootstock's ability to suppress viral replication and not due to low virus titers in the scion. We also confirmed that the choice of rootstock does not affect GFLV levels in a scion, even when comparing scions grafted on rootstocks that can confer tolerance and scions grafted on rootstocks that cannot confer tolerance. This was previously observed by Walker *et al.* (1994b) when ELISA readings of scions grafted on O39-16 and O43-43 were just as high as those grafted on susceptible rootstocks under field conditions.

CONCLUSION

This study is the first to screen GFLV resistance in *M. rotundifolia*-based rootstocks and examine this trait separately from fanleaf degeneration tolerance. O39-16 and O43-43, two rootstocks able to induce fanleaf tolerance to scions, showed remarkably high levels of GFLV resistance, but neither were completely resistant. Both are also highly resistant to *Xiphinema index* (the nematode vector of GFLV). Screening of genotypes from the 07107 population (101-14 x *M. rotundifolia* cv. Trayshed) showed that GFLV resistance is likely a

quantitative trait controlled by multiple genes. Furthermore, we found that GRN-1 does have some degree of GFLV resistance, but it remains to be seen if this will translate into the ability to confer fanleaf tolerance to scions. Although the high GFLV resistance observed in O39-16 and O43-43 seems to indicate a possible correlation between virus resistance and the ability to induce disease tolerance to scions, fanleaf disease field studies utilizing rootstocks from the 07107 population will be key in determining if any correlation exists.

TABLES

Table 1.1. Number of surviving plants after grafting 15 biological replicates.

Rootstock Genotype	Number of Surviving Grafts
07107-002	11
07107-003	1
07107-004	1
07107-005	2
07107-006	11
07107-007	15
07107-008	7
07107-009	2
07107-011	6
07107-012	15
07107-018	15
07107-019	0
07107-032	6
07107-043	14
07107-044	0
07107-046	10
07107-047	10
07107-051	11
07107-056	7
07107-058	9
07107-060	2
07107-062	10
07107-065	9
07107-068	15
07107-069	7
07107-070	12
07107-077	15
07107-091	15
07107-102	12
07107-104	15
07107-105	11
07107-106	9
07107-107	0
07107-108	11
07107-109	2
07107-110	15

07107-112	15
07107-117	1
07107-118	7
07107-120	7
07107-123	1
07107-125	6
07107-129	0
07107-131	1
07107-132	2
07107-133	14
07107-134	3
07107-135	14
07107-147	8
07107-148	14
GRN-1	15
O39-16	15
O43-43	14
St. George	15
101-14	15
Almeria	15
Cabernet Sauvignon	15

Table 1.2. Rootstock genotypes tested for resistance to GFLV six months based on RT-qPCR after being grafted to a GFLV-infected scion. Genotypes are in ascending order of mean $2^{-\Delta C_T}$. Numbers in parentheses represent 95% confidence intervals. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

Rootstock Genotype	$2^{-\Delta C_T}$	Tukey's HSD Result
07107-065	0.00015 (0.00001, 0.00048)	a
O39-16	0.00038 (0.00010, 0.00086)	a
07107-002	0.00088 (0.00040, 0.00155)	ab
O43-43	0.00116 (0.00059, 0.00191)	abc
07107-133	0.00155 (0.00088, 0.00240)	abcd
07107-108	0.00232 (0.00148, 0.00335)	bcde
07107-091	0.00257 (0.00168, 0.00365)	bcdef
07107-008	0.00266 (0.00176, 0.00376)	bcdef
07107-056	0.00271 (0.00179, 0.00381)	bcdef
07107-135	0.00305 (0.00207, 0.00422)	bcdefg
07107-007	0.00327 (0.00225, 0.00447)	bcdefg
07107-120	0.00351 (0.00245, 0.00475)	cdefgh
07107-112	0.00359 (0.00252, 0.00484)	cdefgh
07107-043	0.00377 (0.00267, 0.00505)	cdefgh
07107-105	0.00381 (0.00271, 0.00510)	cdefgh
07107-077	0.00383 (0.00272, 0.00512)	cdefgh
GRN-1	0.00408 (0.00293, 0.00541)	defgh
07107-011	0.00413 (0.00298, 0.00547)	defgh
07107-006	0.00418 (0.00302, 0.00553)	defgh
07107-062	0.00423 (0.00307, 0.00559)	defgh
07107-032	0.00449 (0.00328, 0.00588)	defghi
07107-118	0.00452 (0.00331, 0.00592)	defghij
07107-106	0.00498 (0.00371, 0.00644)	efghijk
07107-069	0.00566 (0.00430, 0.00721)	efghijkl
07107-068	0.00577 (0.00439, 0.00733)	efghijkl
07107-047	0.00586 (0.00447, 0.00744)	fghijkl
07107-018	0.00659 (0.00511, 0.00826)	ghijklm
Cab Sauv	0.00749 (0.00591, 0.00926)	hijklmn
07107-012	0.00897 (0.00723, 0.01090)	ijklmn
07107-051	0.00904 (0.00729, 0.01098)	jklmn
07107-125	0.00944 (0.00765, 0.01141)	klmn
07107-102	0.00949 (0.00770, 0.01147)	klmn
07107-046	0.00951 (0.00771, 0.01149)	klmn
07107-104	0.01000 (0.00816, 0.01203)	lmno
07107-147	0.01015 (0.00829, 0.01219)	lmno
07107-058	0.01056 (0.00867, 0.01265)	lmno

07107-134	0.01102 (0.00908, 0.01315)	mno
07107-148	0.01134 (0.00937, 0.01350)	mno
101-14	0.01202 (0.00999, 0.01424)	no
Almeria	0.01578 (0.01344, 0.01831)	op
07107-110	0.01593 (0.01358, 0.01848)	op
07107-070	0.01960 (0.01698, 0.02241)	p
St. George	0.02092 (0.01821, 0.02382)	p

FIGURES

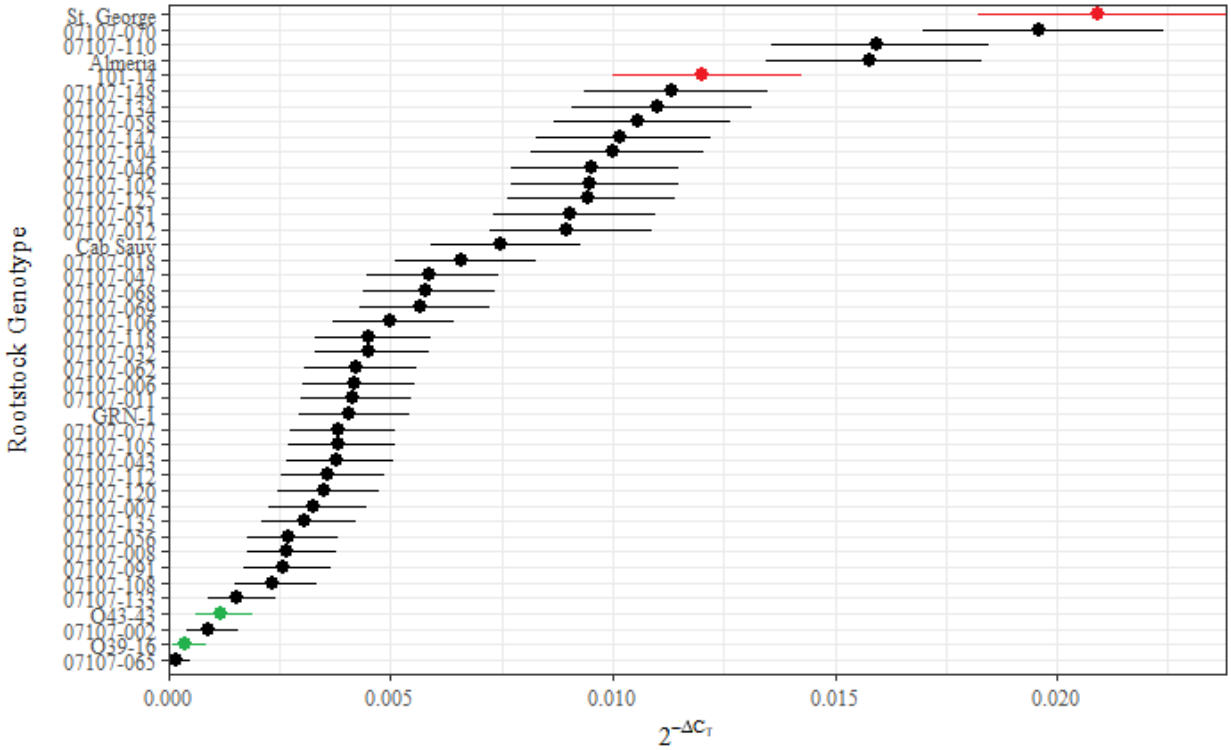

Figure 1.1. Results of RT-qPCR expressed relative to 18S rRNA used as an internal control. Values represent $2^{-\Delta C}{}_T \pm 95\%$ confidence intervals. The susceptible control rootstocks (St. George and 101-14) are represented in red and the tolerant control rootstocks (O39-16 and O43-43) are represented in green.

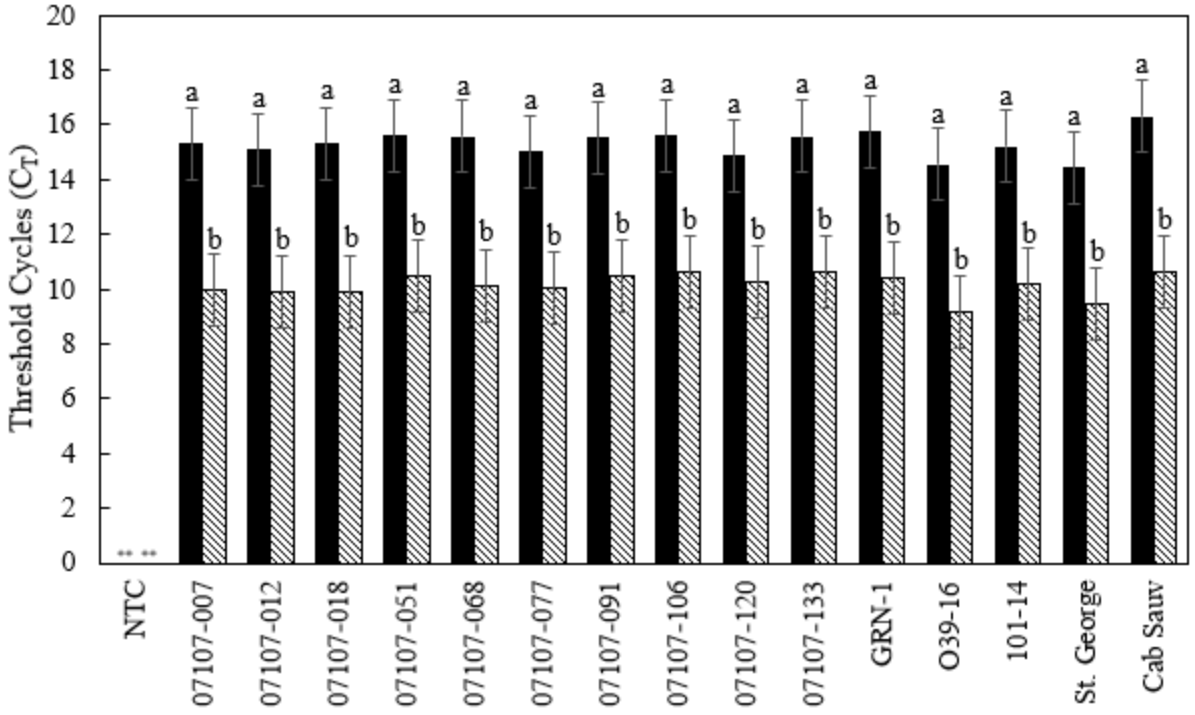

Figure 1.2. Detection of GFLV in scion material grafted to the indicated rootstocks. Values represent mean C_T values ± 95% confidence intervals. Solid black color bars (■) indicate GFLV and dashed bars (▨) indicate the 18S rRNA used as the housekeeping gene. Letters indicate significant differences according to Tukey's test (alpha = 0.05). NTC, no template control; **, undetectable.

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Chapter II.

Grapevine fanleaf degeneration tolerance in a 101-14 Mgt. x *Muscadinia rotundifolia* breeding population

ABSTRACT

One of the most destructive grapevine viruses is grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration. This virus is vectored from root-to-root by the dagger nematode (*Xiphinema index*) and can cause crop losses of up to 80% as a result of the viruses' negative impact on fruit set. Currently, fanleaf degeneration is controlled by grafting vines onto the rootstock O39-16, which prevents the disruption of fruit set. However, breeding efforts to produce alternative fanleaf degeneration rootstocks should continue due to O39-16's *Vitis vinifera* background, which casts doubt on its long-term resistance to grape phylloxera. The objective of this work was to quantify the degree of fanleaf degeneration tolerance that selected progeny from a 101-14 Mgt. x *Muscadinia rotundifolia* cv. Trayshed population conferred to GFLV-infected *V. vinifera* cv. Cabernet Sauvignon scions, and to identify individuals that show promise as rootstocks capable of controlling fanleaf degeneration. A novel method was developed utilizing digital imaging to obtain fruit set ratios for individual clusters as a means to quantify fanleaf tolerance in vines. Forty-one progeny from this hybrid population were grafted with GFLV-infected Cabernet Sauvignon buds and 20 exhibited fruit set ratios that were statistically higher than those of infected vines grafted on the susceptible control rootstocks. Six of these 20 (07107-012, 07107-043, 07107-091, 07107-112, 07107-135, and 07107-148) were able to control fanleaf degeneration as well as O39-16 and exhibited desirable viticultural traits.

INTRODUCTION

Grapevines (*Vitis* spp.) are a major international crop with a strong socioeconomic significance. However, there are an estimated 70 reported viruses that can cause major damage to grapevines, usually reducing productivity and lifespans of vineyards (Martelli, 2014). One such virus is grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration. Commonly known as simply "fanleaf," this disorder can be identified by patches of yellow coloration on the leaves in the spring followed by yellow banding on the leaf veins. Infected vines also show distorted leaves that resemble a fan and have sharp teeth (Andret-Link et al., 2004a). More importantly, fanleaf degeneration can result in crop losses of up to 80% by greatly reducing fruit set (Andret-Link et al., 2004a). Fruit set is defined as the process of a flower forming a berry, and a fruit set ratio can be calculated by dividing the number of berries in a cluster by the total number of flowers in the preceding inflorescence (Roubelakis & Kliewer, 1976). The current measure to control fanleaf degeneration is the usage of the rootstock O39-16 (Walker et al., 1991). This rootstock is a *Vitis vinifera* cv. Almeria x *Muscadinia rotundifolia* cv. Male No. 1 hybrid. Scions grafted on this rootstock do not show the poor fruit set symptoms typically associated with GFLV infection (Walker et al., 1994b).

The virus is vectored by the dagger nematode, *Xiphinema index* (Andret-Link et al., 2004b). Although O39-16 is resistant to the feeding of *X. index*, the nematode can still vector the virus while probing for a feeding site. GFLV moves through the vine and replicates in the scion, but the fruit set on these infected vines is not affected (Walker et al., 1994b).

Although O39-16 has remained effective in the control of fanleaf degeneration since its release in 1988 (Walker et al., 1991), the rootstock has several potential problems. Its *V.*

vinifera background raises concerns regarding its long-term resistance to grape phylloxera (Walker et al., 1994a). O39-16 is also susceptible to root-knot nematodes, which can cause major root damage (Nicol et al., 1999, Walker et al., 1994b). Additionally, O39-16 induces high vigor in scions, which can result in low quality fruit on certain sites (Kliewer & Dokoozlian, 2005, Walker et al., 1994b). Due to these characteristics, usage of O39-16 is typically restricted to fanleaf degeneration sites.

Due to these concerns, breeding efforts for rootstocks capable of inducing fanleaf degeneration tolerance have continued. In 2007, 101-14 Mgt. was crossed with *M. rotundifolia* cv. Trayshed and the resulting progeny have been growing at the University of California, Davis. *Muscadinia rotundifolia* is the source of rootstock-induced tolerance observed in O39-16, and 101-14 Mgt. (*V. riparia* x *V. rupestris*) is a popular commercial rootstock commonly chosen for its ease of propagation, moderate nematode and phylloxera resistance, and the ability to control vigor to scions grafted on it (Bettiga et al., 2003, Walker & Jin, 1998). This cross (notably without any *V. vinifera* parentage) could generate new rootstock alternatives that can induce fanleaf degeneration tolerance to scions without risk of phylloxera susceptibility, while being more easily rooted and grafted.

There have been no published studies exploring a possible correlation between GFLV resistance in a rootstock and its ability to confer fanleaf degeneration tolerance to scions. Resistance is the plant's ability to suppress virus multiplication to a degree (either completely or partially), and tolerance is the ability to lessen the damage caused by virus infection (Oliver & Fuchs, 2011). In Chapter 1, the GFLV resistance of O39-16, St. George (*V. rupestris*), 101-14 Mgt., and selected individuals from the 101-14 Mgt. x *M. rotundifolia* cv. Trayshed population was quantified. The high level of GFLV resistance observed in O39-

16 may hint at a possible correlation between a rootstock's GFLV resistance and its ability to confer fanleaf degeneration tolerance to scions. In this study, this correlation was further explored using select individuals from the 101-14 Mgt. x *M. rotundifolia* cv. Trayshed population.

The goal of this study was to quantify the degree of fanleaf degeneration tolerance that selected individuals from the 101-14 Mgt. x *M. rotundifolia* cv. Trayshed population can confer to GFLV-infected *V. vinifera* cv. Cabernet Sauvignon scions. The fruit set ratio of infected vines grafted on rootstocks from the 101-14 Mgt. x *M. rotundifolia* cv. Trayshed population was measured, and these values were compared to those of uninfected vines and infected vines grafted on known susceptible and tolerant control rootstocks. This study presents findings on the inheritance of the ability to confer fanleaf degeneration tolerance to scions and identifies six genotypes (07107-012, 07107-043, 07107-091, 07107-112, 07107-135, and 07107-148) that show promise as potential rootstocks capable of controlling fanleaf degeneration.

MATERIALS AND METHODS

Plant Material

All GFLV-infected scion material (*V. vinifera* cv. Cabernet Sauvignon) was collected from a vineyard in Rutherford, CA. Its sole infection with GFLV was verified by RT-qPCR at the Virus Identification Lab of Foundation Plant Services (FPS) at UC Davis.

Rootstock material for this study was obtained from a virus-tested research vineyard at the University of California, Davis. Crosses of 101-14 Mgt. x *M. rotundifolia* cv. Trayshed were made in 2007 and the resulting progeny were grown in a vineyard free of *X. index*. This

population will hereby be referred to as the 07107 population. There are 85 surviving individuals in this population and 50 of those genotypes were selected for the study. The other 35 individuals in this population were too weak to provide adequate dormant cuttings, so they were excluded from the available pool of plants.

The tolerant control rootstock (O39-16) and the susceptible control rootstocks (St. George and 101-14) were FPS certified and virus-tested. Virus-free *V. vinifera* cv. Cabernet Sauvignon was also obtained from FPS to act as scions for the uninfected controls.

Grafting

In January 2017, dormant cuttings (about 0.5-1 cm in diameter) were taken for bench grafting and stored at 4ºC until used. Lengths of cuttings, preparation of cuttings, and grafting methods are described in Chapter 1. All rootstocks were grafted with the GFLV-infected scion material described above. The tolerant control rootstock (O39-16) and the susceptible control rootstocks (St. George and 101-14) were also grafted with virus-free Cabernet Sauvignon scions to act as virus-free controls. Virus-free Cabernet Sauvignon was also grafted with itself to provide additional virus-free controls. No genotypes from the 07107 population were grafted with virus-free Cabernet Sauvignon scions. Bench grafts were callused and treated as described in Chapter 1 to induce root formation.

Plant Growth

Bench grafts that had rooted and callused were grown in a greenhouse before being transferred to the field. Temperature in the greenhouse was set to 30ºC/20ºC day/night and

the photoperiod was maintained at 16 hours with supplemental lighting. In the greenhouse, plants were watered every two days with a modified Hoagland's solution.

Five replicates of each graft combination were transferred to the field in May 2017 and planted in a randomized design. Vine spacing was 2.1 m x 2.7 m (vine by row). The vineyard was free of *X. index*. Vines were trained to unilateral cordons and spur pruned. The vines were allowed to flower and fruit the year after they were planted. Pruning weight was recorded at the end of the second and third growth cycles (January 2019 and January 2020, respectively).

Fruit Set Quantification

Fruit set quantification started during the 2018 growing season and continued for two additional years afterwards. In early May, white sheer fabric bags (commonly known as organza bags) were tied around two randomly chosen inflorescences on each vine. These bags allowed free air circulation and admitted light but prevented the loss of abscised flowers. The bags were rectangular, measuring 12.7 cm x 17.8 cm. Bags were tied in a way such that when the flowers bloomed, all calyptras would be captured and preserved inside the bags (Figure 2.1). Weak vines with limited growth and no inflorescences were noted but not bagged.

In July, all bags were harvested with the cluster and loose calyptras still inside each bag (Figure 2.2). A label was inserted into each bag to indicate which vine the bag was harvested from. Harvested bags were stored at 4^{o}C.

Within two weeks after harvest, all grape clusters from the sheer bags were transferred into individual plastic bags labeled with the original vine the cluster originated

from. These clusters were stored at 4ºC. Calyptras were kept in the sheer bags and stored at room temperature after separation from the cluster. Care was taken to ensure all calyptras were brushed and shaken off the cluster and then returned to the sheer bag during the separation process.

Within two weeks after separation from the calyptras, all berries from each respective cluster were removed from the rachis and placed on a digital scanner (Epson Perfection V39), one cluster at a time. All berries from each respective cluster were scanned and digitally captured into an image, resulting in individual image files that each depicted all the berries for each cluster (Figure 2.3). Care was taken to ensure that there was a monolayer of berries on the scanner so that the resulting image would accurately capture every berry. Berries were scanned in greyscale at 300 dpi. The image files were processed with a batch processing macro in ImageJ to isolate and count the number of berries present in each image, resulting in a berry count for each cluster.

Calyptras from each respective cluster were also scanned and digitally captured into an image. As with the berries, all calyptras from each respective cluster were scanned and digitally captured into an image, resulting in individual image files that each depicted all the calyptras for each cluster (Figure 2.4). Due to the small size of the calyptras, a paintbrush was used to spread the calyptra mixture across the surface of the scanner to ensure minimal clumping. Visible debris present in the calyptra mixture (such as dried anthers) was acceptable for the image scan. Calyptras were scanned in color at 600 dpi. The image files were processed with a batch processing macro in ImageJ to isolate and count the number of calyptras present in each image and then output a calyptra count for each cluster. The software was given criteria that allowed it to consider the size, color, and shape of each

object or cluster of objects to identify calyptras or clumps of calyptras (Figure 2.5). Clumps of calyptras were successfully identified and counted accordingly in the macro.

Fruit set was quantified for each cluster by dividing the berry count of the cluster by the cluster's calyptra count.

Statistical Analysis

An analysis of variance (ANOVA) was used to determine the effects of rootstock genotype, harvest year (2018, 2019, and 2020), and their interactions on fruit set. The Anderson-Darling test, Levene's test, and outlier test were used, respectively, to test for assumptions of normality, equality of variances, and outliers. A $\log_{10}$ transformation was applied to the fruit set values to meet parametric assumptions. ANOVA was performed in RStudio v1.3.1056 running R v4.0.2 with the *emmeans* package, with full interaction and all effects considered as fixed effects. Means presented are back transformed from transformed data. Tukey's test was performed for pairwise means comparisons.

An ANOVA was also used to determine the effects of rootstock genotype, year (2019 and 2020), and their interactions on pruning weight of the vines. Normality, homogeneity of variances, and outliers were evaluated using the Anderson-Darling Test, Levene's Test, and outlier test, respectively. Values were square-root transformed to meet parametric assumptions. ANOVA was performed in RStudio v1.3.1056 running R v4.0.2 with the *emmeans* package, with full interaction and all effects considered as fixed effects. Due to the strong effect of time, an ANOVA for each year was used to determine the effects of rootstock genotype on pruning weight. To meet assumptions of ANOVA, a $\log_{10}(1+x)$

transformation was applied to pruning weight values. Tukey's test was performed for pairwise means comparisons.

RESULTS

Vine Survival Rate

From the 50 rootstock genotypes selected from the 07107 population for this study, 41 genotypes survived with five or more biological replicates from the original fifteen bench grafts. The nine rootstock genotypes with less than five surviving biological replicates were discarded from the study. These nine genotypes were 07107-003, 07107-004, 07107-019, 07107-044, 07107-060, 07107-107, 07107-123, 07107-129, and 07107-131.

Vine survival rates after three growing cycles in the field are presented in Table 2.1. Notably, even though vines grafted on 07107-065 and 07107-110 had a 60% and 80% survival rate respectively, all surviving vines from these rootstock genotypes are weak and show very low vigor.

Vine Pruning Weight

Significant differences in pruning weight among rootstock genotypes were observed ($P < 0.01$) within each year that pruning weight was recorded. To interpret differences among genotypes, Tukey's test was performed. Uninfected vines grafted on St. George had the highest pruning weight at the end of the second growth cycle (January 2019). All vines (both infected and uninfected) grafted on control rootstocks (101-14, St. George, O39-16, and Cabernet Sauvignon) were statistically similar to each other in terms of pruning weight at the end of the second growth cycle. During this period, vines grafted on 07107-011 had the

lowest pruning weight. This genotype, as well as vines grafted on 07107-065 and 07107-110, had statistically lower pruning weights than uninfected vines grafted on St. George, but vines grafted on these three 07107 rootstock genotypes had statistically similar pruning weights to all other controls. All other vines grafted on rootstock genotypes from the 07107 population had statistically similar pruning weights to all vines on control rootstocks.

At the end of the third growth cycle (January 2020), uninfected vines grafted on Cabernet Sauvignon had the highest pruning weight, and all vines grafted on control rootstocks were again statistically similar to each other in terms of pruning weight. Vines grafted on 07107-011, 07107-047, 07107-065, 07107-110, and 07107-148 had statistically lower pruning weights than all uninfected controls. Vines grafted on 07107-011 had the lowest pruning weight for the second year in a row. All other vines grafted on 07107 rootstocks had statistically similar pruning weights as uninfected vines grafted on O39-16 and 101-14.

Fruit Set Quantification

Significant differences in fruit set among rootstock genotypes were observed ($P < 0.001$). ANOVA determined that harvest year had no influence on fruit set. To interpret differences among genotypes, Tukey's test was performed (Table 2.2). Infected vines grafted on 101-14 had the lowest fruit set. Infected vines grafted on St. George, as well as vines grafted on seventeen genotypes from the 07107 population, had fruit set ratios that were statistically similar as infected vines on 101-14. Vines grafted on three genotypes from the 07107 population (07107-032, 07107-109, 07107-110) had statistically higher fruit set than

infected vines on 101-14, but these three genotypes were statistically similar to infected vines on St. George.

All uninfected controls were statistically similar to each other and had fruit set ratios that were statistically higher than the susceptible infected controls (infected vines grafted on St. George and 101-14). Infected vines on O39-16 were not statistically different from the uninfected controls and had statistically higher fruit set than the infected susceptible controls. Vines grafted on 07107-043 had the highest fruit set ratio in the study and were statistically similar to all uninfected controls as well as infected vines on O39-16. Other than 07107-043, vines grafted on nineteen other genotypes from the 07107 population had statistically higher fruit set ratios than both infected susceptible controls.

Plotting the mean fruit set ratios for scions grafted on each rootstock genotype shows that the ability to induce fanleaf degeneration tolerance to GFLV-infected scions did segregate in 07107 population, but the trait appears to be quantitatively controlled because of the wide range and distribution of tolerance-inducing levels in the population (Figure 2.6).

DISCUSSION

Due to the *V. vinifera* background of O39-16, it is recommended that this rootstock should be considered an interim solution to fanleaf degeneration and that breeding should continue for a more durably resistant rootstock without *V. vinifera* in its background. O39-16 is already known to be susceptible to root-knot nematode, and its *V. vinifera* background raises concerns regarding its long-term resistance to grape phylloxera (Walker et al., 1994a). However, it has been planted in commercial vineyards for 30 years without collapsing to phylloxera. Its sibling, O43-43, was released at the same time and collapsed to phylloxera in

two years (Walker et al., 2014). There have been few studies regarding GFLV resistance and fanleaf degeneration tolerance, with most of the current studies incorporating GFLV resistance through genetic engineering (Hemmer et al., 2018, Pakbaz et al., 2018). There have been no published studies that have extensively quantified fruit set as a method to determine the degree of fanleaf degeneration tolerance induced by a rootstock to GFLV-infected scions. The present study developed a novel method utilizing fruit set ratios to quantify fanleaf degeneration tolerance and applied it to a promising breeding population composed of progeny from a cross of 101-14 Mgt. and *M. rotundifolia* cv. Trayshed.

Muscadinia rotundifolia is known to be difficult to root from dormant cuttings and it generally forms incompatible unions when grafted to *V. vinifera* cultivars (Bouquet, 1980). Therefore, when considering the 07107 population (101-14 x *M. rotundifolia* cv. Trayshed) as a rootstock population, it is important to evaluate the level of graft success in the progeny. For industry standards, a success rate of 80% or more is highly preferred. In Chapter 1, graft success rates (determined six weeks after grafting) were presented for selected individuals from the 07107 population. Thirteen genotypes in this previous study (07107-007, 07107-012, 07107-018, 07107-043, 07107-068, 07107-077, 07107-091, 07107-104, 07107-110, 07107-112, 07107-133, 07107-135, and 07107-148) showed promising graft success rates exceeding 80%. In the current study, grafted vine survival rates after three growing seasons in the field are presented and they may be a better indicator of long-term vine performance (Table 2.1). Of the thirteen genotypes listed above, vines that were grafted on eight of these rootstock genotypes (07107-012, 07107-018, 07107-043, 07107-091, 07107-112, 07107-133, 07107-135, and 07107-148) had a 100% survival rate in the field after three growing seasons, and vines grafted on the remaining five genotypes had an 80% survival rate in the field after

three growing seasons. Vine survival should continue to be observed after every growing season to take note of any future vine collapse due to delayed graft incompatibility.

There were few significant differences in pruning weight between rootstock genotypes in 2019. The only notable statistical differences were that vines grafted on 07107-011, 07107-065, and 07107-110 had statistically lower pruning weights than uninfected vines grafted on St. George. The lack of additional noticeable differences may be due to the fact that these vines had only been planted for a year and a half at this point and they were still young and establishing themselves. More differences could be expected as the vines grew older.

As expected, more differences between genotypes in pruning weight were observed in 2020. During this year, vines grafted on 07107-011, 07107-047, 07107-065, 07107-110, and 07107-148 had statistically lower pruning weights than all uninfected controls, which may be the result of lower vigor induced by these rootstock genotypes. A lower vigor alternative to O39-16 would address complaints about the relatively high vigor it induces in scions (Walker et al., 1994b). However, the lower pruning weights and vigor may also indicate that the vines are declining and may collapse in the near future. This collapse is likely for vines grafted on 07107-011 and 07107-047, which have a 20% and 40% survival rate, respectively, after three growing seasons (Table 2.1). Pruning weights should continue to be taken for these vines in future years to detect differences in vigor that may manifest as the vines become more established.

O39-16 controls fanleaf degeneration by preventing the poor fruit set typically associated with GFLV infection (Walker et al., 1994b). In this study, O39-16 performed as expected with GFLV-infected vines grafted on O39-16 having statistically similar levels of

fruit set as uninfected vines and higher levels of fruit set than vines grafted on fanleaf-susceptible rootstocks (Table 2.2). This finding confirms that fanleaf tolerance is induced by O39-16 and counters claims that it has not been conclusively demonstrated (Oliver & Fuchs, 2011). Vines grafted on O39-16 also performed statistically similar as vines grafted on the rootstock with the highest fruit set ratio in the study, 07107-043.

Infected vines grafted on 101-14 Mgt., the maternal parent of the 07107 population, did not have fanleaf tolerance when compared to vines grafted on its offspring (Table 2.2 and Figure 2.6). This validates that fanleaf tolerance in the 07107 population is most likely inherited from the *M. rotundifolia* cv. Trayshed parent. This *M. rotundifolia* cultivar was unable to be included in this study due to the difficulty in grafting *M. rotundifolia* to *Vitis* spp. scions and the inability of *M. rotundifolia* to root from dormant cuttings (Bouquet, 1980).

The 07107 population segregated for the level of fanleaf tolerance conferred to GFLV-infected scions (Figure 2.6). Due to the lack of any distinctive segregation ratio and the presence of a wide gradient of tolerance levels, we conclude that this trait is controlled by multiple genes and inherited quantitatively. The phenotypic data obtained in this study provides a foundation for future efforts to locate a genetic marker for a rootstock's ability to confer fanleaf tolerance to GFLV-infected scions. However, a much larger population size will be required. There are polymorphic markers available for *M. rotundifolia* and an SSR marker-based genetic map of *M. rotundifolia* has been published (Riaz et al., 2012).

Out of the 41 rootstock genotypes tested from the 07107 population, vines grafted on 20 of these genotypes demonstrated fruit set ratios that were statistically higher than those of infected vines grafted on the susceptible control rootstocks (Table 2.2). All twenty of these

rootstock genotypes performed statistically similar to the uninfected control vines as well as infected vines grafted on O39-16, a known tolerant rootstock. Of these 20 genotypes, six genotypes had a 100% survival rate in the field (Table 2.1) as well as demonstrating a graft success rate exceeding 80% in Chapter 1. These six genotypes (07107-012, 07107-043, 07107-091, 07107-112, 07107-135, and 07107-148) show promise as potential rootstocks capable of inducing fanleaf degeneration tolerance to scions as well as exhibiting acceptable graft success and a 100% vine survival rate in this study. Notably, vines grafted on 07107-148 exhibited lower vigor as these vines produced statistically lower pruning weights in 2020 than all uninfected control vines. Due to their promising performance in our studies, the six rootstock genotypes listed above should be screened for resistance against other grapevine diseases and pests, especially phylloxera, to determine their suitability to be used in commercial vineyards.

Chapter 1 presented evidence that O39-16 possesses GFLV resistance since it suppressed replication of GFLV compared to St. George and 101-14. Given that O39-16 can confer fanleaf tolerance, but St. George and 101-14 cannot, a possible correlation between a rootstock's ability to induce fanleaf tolerance and its ability to suppress GFLV replication was sought. However, when comparing the GFLV resistance results of the 07107 population in Chapter 1 with the fruit set ratios of infected vines grafted on these rootstocks, there does not appear to be a relationship between GFLV resistance in a rootstock and that rootstock's ability to induce fanleaf tolerance to infected scions. Further studies need to be designed to more completely test this correlation.

CONCLUSION

This study is the first to have extensively quantified fruit set as a method to determine the degree of fanleaf degeneration tolerance induced by a rootstock to GFLV-infected vines. O39-16 was confirmed to possess the ability to induce fanleaf tolerance to scions since GFLV-infected vines grafted on O39-16 showed statistically similar levels of fruit set as uninfected vines and statistically higher levels of fruit set than vines grafted on fanleaf-susceptible rootstocks. Screening of genotypes from the 07107 population (101-14 x *M. rotundifolia* cv. Trayshed) showed that this tolerance-conferring ability is likely a quantitatively inherited trait controlled by multiple genes. Furthermore, we found that six genotypes from this population (07107-012, 07107-043, 07107-091, 07107-112, 07107-135, and 07107-148) have promise as potential new rootstocks capable of controlling fanleaf degeneration while possessing good grafting ability. Like O39-16, these six genotypes had statistically similar levels of fruit set as uninfected vines and statistically higher levels of fruit set than vines grafted on fanleaf-susceptible rootstocks. Although these rootstocks appear promising in combating fanleaf, additional and longer trials (especially for screening resistance against other grapevine diseases and pests) should be conducted to determine their value as future commercial rootstocks.

TABLES

Table 2.1. Survival rates of *V. vinifera* cv. Cabernet Sauvignon grafted on rootstock genotypes after three growing cycles. Five vines were originally planted in the field.

Survival Rate	Rootstock Genotypes
20%	07107-011
40%	07107-047, 07107-102, 07107-109
60%	07107-032, 07107-065, 07107-134
80%	07107-005, 07107-007, 07107-008, 07107-009, 07107-056, 07107-062, 07107-068, 07107-069, 07107-077, 07107-104, 07107-106, 07107-108, 07107-110, 07107-117, 07107-118, 07107-132
100%	07107-002, 07107-006, 07107-012, 07107-018, 07107-043, 07107-046, 07107-051, 07107-058, 07107-070, 07107-091, 07107-105, 07107-112, 07107-120, 07107-125, 07107-133, 07107-135, 07107-147, 07107-148, 101-14 (Infected), 101-14 (Uninfected), St. George (Infected), St. George (Uninfected), O39-16 (Infected), O39-16 (Uninfected), and Cabernet Sauvignon (Uninfected)

Table 2.2. Fruit set of *V. vinifera* cv. Cabernet Sauvignon grafted on rootstock genotypes. Unless otherwise indicated, all vines are infected with fanleaf degeneration. Genotypes are in descending order of mean fruit set. Numbers in parentheses represent 95% confidence intervals. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

Rootstock Genotype	Fruit Set	Tukey's HSD Result
07107-043	0.595 (0.503, 0.704)	a
07107-112	0.551 (0.474, 0.641)	ab
07107-008	0.520 (0.436, 0.621)	abc
07107-006	0.518 (0.447, 0.600)	abc
07107-012	0.513 (0.437, 0.602)	abc
07107-147	0.509 (0.440, 0.588)	abc
07107-135	0.478 (0.410, 0.557)	abc
07107-062	0.476 (0.402, 0.563)	abc
07107-148	0.472 (0.402, 0.554)	abc
07107-002	0.470 (0.405, 0.544)	abc
07107-009	0.465 (0.392, 0.553)	abcd
07107-091	0.464 (0.394, 0.547)	abcd
07107-070	0.461 (0.397, 0.534)	abcd
07107-058	0.458 (0.395, 0.533)	abcd
07107-046	0.452 (0.382, 0.534)	abcd
St. George (Uninfected)	0.449 (0.392, 0.515)	abcd
07107-120	0.448 (0.383, 0.524)	abcd
07107-125	0.448 (0.380, 0.528)	abcd
O39-16 (Uninfected)	0.448 (0.389, 0.515)	abcd
101-14 (Uninfected)	0.444 (0.386, 0.510)	abcd
O39-16 (Infected)	0.443 (0.386, 0.508)	abcd
Cab Sauv (Uninfected)	0.440 (0.383, 0.506)	abcd
07107-007	0.435 (0.374, 0.505)	abcd
07107-117	0.433 (0.366, 0.512)	abcd
07107-005	0.422 (0.340, 0.524)	abcd
07107-110	0.417 (0.332, 0.523)	abcde
07107-109	0.416 (0.328, 0.528)	abcde
07107-032	0.399 (0.326, 0.488)	abcde
07107-134	0.396 (0.312, 0.502)	abcdef
07107-132	0.385 (0.329, 0.450)	abcdef
07107-018	0.384 (0.330, 0.446)	abcdef
07107-133	0.380 (0.319, 0.451)	abcdef
07107-047	0.371 (0.273, 0.504)	abcdef
07107-106	0.370 (0.313, 0.437)	abcdef
07107-065	0.365 (0.291, 0.458)	abcdef
07107-104	0.356 (0.297, 0.427)	bcdef
07107-068	0.355 (0.294, 0.428)	bcdef
07107-051	0.348 (0.302, 0.402)	cdef

07107-056	0.345 (0.286, 0.416)	cdef
07107-077	0.334 (0.281, 0.397)	cdef
07107-118	0.330 (0.279, 0.390)	cdef
07107-108	0.321 (0.261, 0.396)	cdef
07107-069	0.319 (0.268, 0.379)	cdef
07107-102	0.311 (0.246, 0.395)	cdef
07107-105	0.301 (0.260, 0.349)	def
St. George (Infected)	0.245 (0.213, 0.281)	ef
101-14 (Infected)	0.232 (0.198, 0.272)	f

FIGURES

Figure 2.1. Bagging of inflorescences pre-bloom in order to capture calyptras for counting.

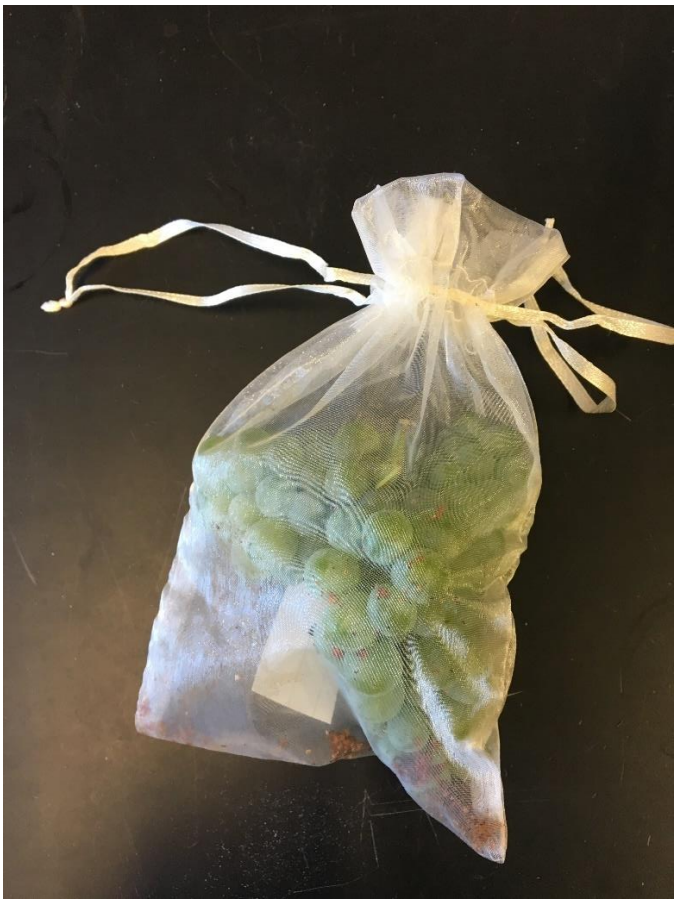

Figure 2.2. Sheer bags after harvesting. Notice the dried calyptras at the bottom of the bag.

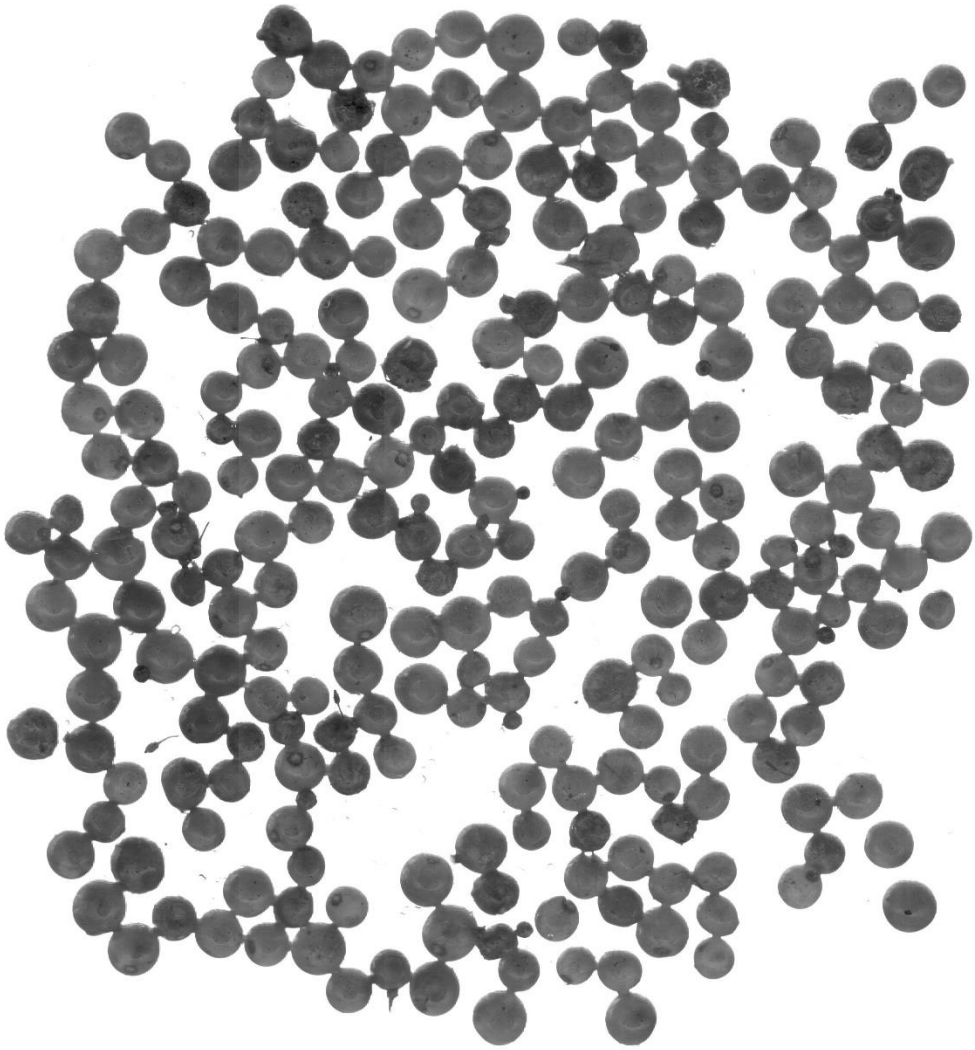

Figure 2.3. Example scan of all berries from a single cluster. (Image taken from GFLV-infected Cabernet Sauvignon grafted on 07107-058.)

Figure 2.4. Example scan of all calyptras (and other dried flower parts) collected from a single inflorescence. (Image taken from GFLV-infected Cabernet Sauvignon grafted on 07107-105.)

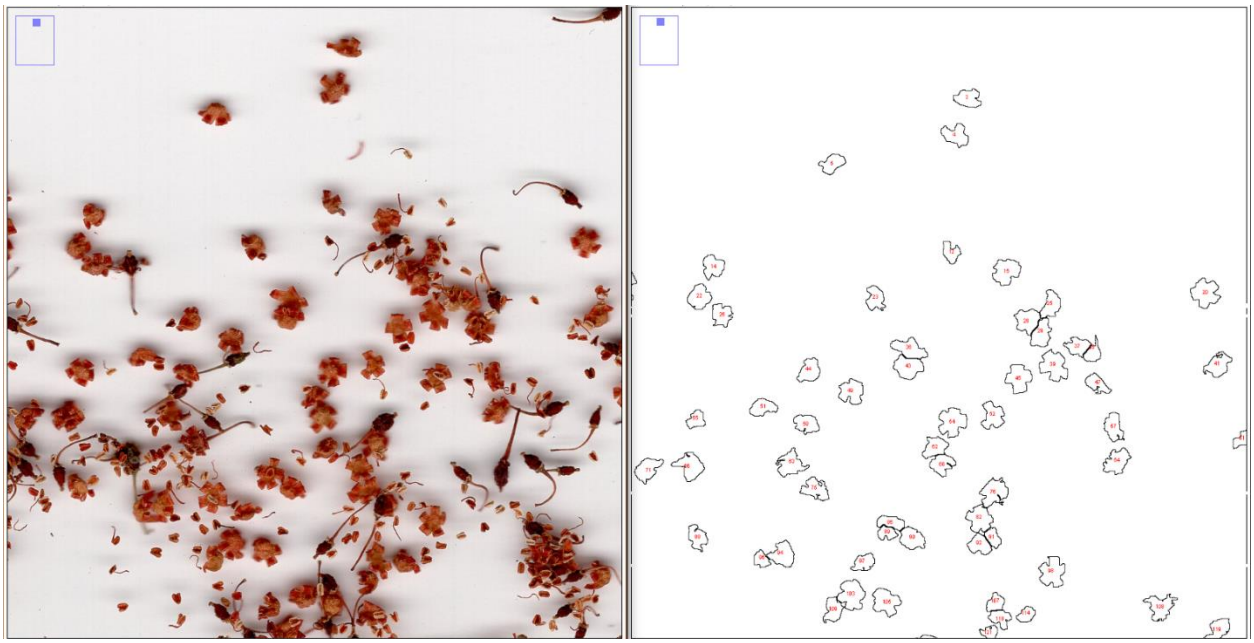

Figure 2.5. Side-by-side comparison of an unedited scan of calyptras (left) and ImageJ applying criteria to identify and isolate calyptras (right). (Image taken from uninfected Cabernet Sauvignon grafted on St. George.)

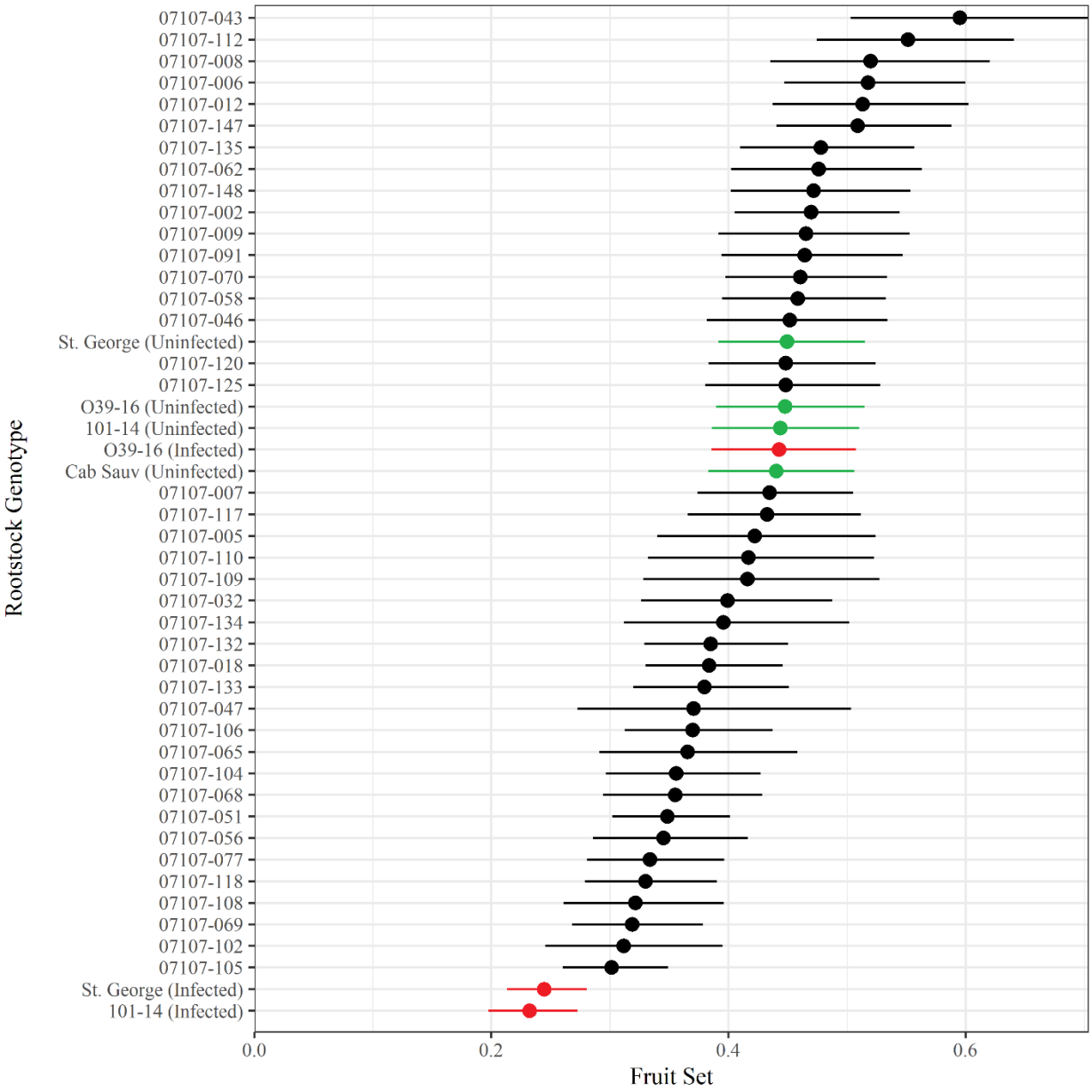

Figure 2.6. Fruit set ratios of *V. vinifera* cv. Cabernet Sauvignon grafted on rootstock genotypes from the 07107 (101-14 x *M. rotundifolia* 'Trayshed') population. Unless otherwise indicated, all vines are infected with fanleaf degeneration. Values represent mean fruit set ratio ± 95% confidence intervals. Infected controls are represented in red and uninfected controls are represented in green.

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Chapter III.

Performance of nematode-resistant rootstocks on a fanleaf site in the San Joaquin Valley

ABSTRACT

Nematodes are a major problem in the grape-growing industry, particularly *Xiphinema index* due to its vectoring of grapevine fanleaf virus, the causal agent of fanleaf degeneration. Because eradicating nematodes from vineyard soil has been unsuccessful, control efforts have focused on breeding new resistant rootstocks. In a response to the needs of California's grape-growing regions, five rootstocks with broad and durable nematode resistance were released in 2008. These five rootstocks (GRN-1, GRN-2, GRN-3, GRN-4, and GRN-5) possess resistance to *X. index* and may be promising alternatives to O39-16, currently the only commercially available fanleaf resistant rootstock. The objective of this work was to evaluate the viticultural characteristics of these new rootstocks on a fanleaf site in the San Joaquin Valley in California. The GRN series was compared with several rootstock standards, such as O39-16, to determine their suitability on fanleaf sites. All five GRN rootstocks performed comparably to O39-16 in terms of vine performance during the three growing seasons evaluated for this study. GRN-1 showed notable promise due to its low GFLV infection rate on the site. Although further studies are needed to evaluate the long-term performance of these rootstocks, these initial results are promising.

INTRODUCTION

Nematodes, particularly dagger (*Xiphinema index*), root-knot (*Meloidogyne* sp.), ring (*Mesocriconema xenoplax*), and root lesion (*Pratylenchus vulnus*), pose serious challenges for the grape production industry. These soil-borne pests damage roots and prevent new root development, usually leading to weak vines and reduced yields and may result in eventual death (Bridge & Starr, 2007). The cumulative effect over years causes significant losses and notable economic damage (Nicol et al., 1999). Certain nematodes can also vector harmful viruses, such as grapevine fanleaf virus and tomato ringspot virus, that cause further damage to the vine (Martelli, 2014).

The usage of nematicides to combat these soil-borne pests is restricted due to the high toxicity and damage to the environment caused by the chemicals (Van Zyl et al., 2012, Villate et al., 2012). Crop rotation and fallow periods are costly due to the high premium placed on vineyard land and the long fallow period required (six to ten years) for elimination of nematodes (Demangeat et al., 2005). Therefore, breeding efforts have focused on combating the pests by developing new resistant rootstocks (Ferris et al., 2012).

Five nematode-resistant rootstocks ('UCD GRN-1,' 'UCD GRN-2,' 'UCD GRN-3,' 'UCD GRN-4,' and 'UCD GRN-5') were released by the University of California in 2008 (Ferris et al., 2012). These rootstocks were developed and selected for their broad and durable nematode resistance, as well as possessing horticultural characteristics selected for grape-growing regions in California. All five rootstocks in the GRN (Grape Rootstock for Nematodes) series have been shown to resist root-knot nematode (*Meloidogyne incognita* Race 3, *M. incognita* pathotype Harmony C, and *Meloidogyne arenaria* pathotype Harmony A) and dagger nematode (*Xiphinema index*) singly and in a combined inoculum. Their

resistance to root-knot nematodes has also been tested in warm soils (30 ºC) where root-knot nematode resistance fails. GRN-1 (*Vitis rupestris* A. de Serres x *Muscadinia rotundifolia* cv. Cowart) has been previously described and studied in Chapter 1 for its potential to possess grapevine fanleaf virus (GFLV) resistance. Notably, this rootstock possesses a *M. rotundifolia* background while lacking *V. vinifera* parentage, thus alleviating fears regarding its grape phylloxera susceptibility. Additional information regarding GRN-1 and the other rootstocks in the GRN series (such as parentage and host status to various nematodes) is detailed in Ferris *et al.* (2012).

One of the most destructive nematodes found in vineyards around the world is the dagger nematode *X. index,* which is widespread in California and is particularly common in the northern San Joaquin Valley (Ferris & McKenry, 1976). This nematode is the vector of grapevine fanleaf virus (GFLV), the causal agent of fanleaf degeneration (Hewitt et al., 1958). The disease can result in crop losses of up to 80% by greatly reducing fruit set and causing formation of 'shot berries,' small, seedless berries that do not mature (Andret-Link et al., 2004). The current measure to control fanleaf degeneration is usage of the rootstock O39-16 (*V. vinifera* cv. Almeria x *M. rotundifolia* cv. 'Male No. 1') (Walker et al., 1991). Despite the rootstock's resistance to *X. index,* the nematode can still vector the virus while probing for a feeding site (Walker et al., 1994b). However, even after GFLV spreads up the rootstock and into the scion (where it attains high titers), the disruption of fruit set usually accompanied by fanleaf infection is not observed in scions grafted on O39-16 (Walker et al., 1994b). Despite the effectiveness of O39-16 in inducing fanleaf degeneration tolerance to vines, the rootstock has several potential problems which have been previously described in Chapters 1 and 2, such as its susceptibility to root-knot nematodes and its *V. vinifera*

background raising concerns regarding its long-term resistance to grape phylloxera (Nicol et al., 1999, Walker et al., 1994a, Walker et al., 1994b). Due to these concerns, it is important to develop more rootstocks that can perform satisfactorily on fanleaf degeneration sites. The GRN rootstocks, particularly GRN-1 with its *M. rotundifolia* background, are promising potential alternatives.

Although the GRN rootstocks are resistant to *X. index* feeding, it is unknown if GFLV vectoring is still possible, such as the case in O39-16. Additionally, due to the relatively recent release of the GRN rootstocks, the overall performance of these rootstocks on a vineyard site infested with GFLV has not been evaluated.

The objective of this study was to evaluate the viticultural characteristics of the GRN rootstocks on a fanleaf site in the San Joaquin Valley. The GRN series was compared with several rootstock standards by quantifying their performance and determining their influence on vine productivity. The GFLV infection status of individual vines was also determined to evaluate the severity of infection on the site and the performance of the GRN rootstocks against GFLV infection compared to standard rootstocks.

MATERIALS AND METHODS

Site and Establishment

The study was established in 2011 in Acampo, CA as part of the Lodi Liberty Rootstock Trial. *Vitis vinifera* cv. Malbec scions were bench-grafted to GRN-1, GRN-2, GRN-3, GRN-4, GRN-5, 101-14, 1103P, 3309C, Harmony, O39-16, RS-3, RS-9, and St. George rootstocks and planted in the vineyard according to the experimental design. 101-14, 1103P, 3309C, and Harmony were included in the trial because they are commonly used

rootstocks in California. O39-16 is known to induce fanleaf tolerance to scions, and St. George is known to be highly susceptible to *X. index* and GFLV. RS-3 and RS-9 are nematode-resistant rootstocks that were released in 2003. The study was established in a completely randomized block design, with 13 rootstock treatments and five single-row blocks (plots) of each rootstock (Figure 3.1). Each plot contained five adjacent vines of the same rootstock. Because vines grafted on St. George are known to be susceptible to fanleaf degeneration, additional vines grafted on St. George were planted at the row ends and between blocks as a measure to gauge overall distribution and fanleaf severity at the site.

The soil was a Tokay fine sandy loam that had 0 to 2 percent slopes. The region has a Mediterranean climate with an average minimum winter temperature of 3.8 ^{o}C and average maximum summer temperature of 32.3 ^{o}C, and precipitation averaging 483 mm annually (National Oceanic and Atmospheric Administration, 2021). The rootzone depth was unknown but believed to be deep. Vine spacing was 5 ft x 11 ft (vine by row). The trellis system was a quadrilateral system with crossarms above the cordon to catch shoots. The cover crop used was a permanent sod of perennial rye. Before vineyard establishment, nematode analysis showed *X. index*, pin nematodes, root-knot nematode larvae, and ring nematodes present on the site (Western Diagnostic Services, Clarksburg, CA).

Vineyard Management

Drip irrigation was used to supplement rainfall with irrigation scheduling following Best Management Practices (BMP) for the Lodi region. Irrigation followed a moderate Regulated Deficit Irrigation (RDI) program of 80% estimated ETc from berry set to veraison (Prichard et al., 2004). During the post-harvest period, vineyard irrigation was increased to

100% ETc. Fertilization included 30-40 lbs actual N/acre and 60 lbs actual K/acre applied post bloom annually. Zn and Mg were applied as needed based on petiole results. Pest management followed University of California Integrated Pest Management (UC IPM) recommendations and customary practices for the Lodi region (Haviland et al., 2016).

Dormant vines were pruned to retain 30-40 two-node spurs based on vine size and health. Canopy management practices used were consistent with regional guidelines and included shoot thinning and leaf removal (Dokoozlian, 2009). Shoot thinning was performed pre-bloom and consisted of removal of non-count shoots (shoots not originating from spur positions). Leaf removal was performed at berry set only on the north side of the vine. Four to six leaves were removed to open a window in the fruiting zone. All fruit was hand-harvested with yields determined at maturity as defined by standard metrics such as pH and titratable acidity.

Data Collection

All fruit from the trial was harvested during the 2014, 2018, and 2020 growing seasons to determine total fruit yield and number of clusters per vine. Random ripe berries from each plot were collected, refrigerated, and analyzed within two days for titratable acidity using standard methods. Pruning weight data were collected in February of the following year (2015, 2019, and 2021) to quantify vegetative growth during the previous year. GFLV infection status was determined by ELISA for vines grafted on GRN-1, GRN-2, GRN-3, GRN-4, GRN-5, 1103P, O39-16, and St. George (Agdia, Inc.). ELISA was conducted on callus tissue obtained from hardwood cuttings collected in January 2021. An

ELISA sample was considered to be negative if its OD_{405} value was less than two times the negative control value.

Statistical Analysis

All data were subject to an analysis of variance (ANOVA) to determine the differences among rootstocks in fruit production, fruit characteristics, and vine size for each growing season. ANOVA was performed in RStudio v1.3.1056 running R v4.0.2 with the *emmeans* package. Tukey's test was performed for pairwise means comparisons. The Jenks natural breaks classification method was used to determine breakpoints in the ELISA results to classify vines that tested positive as either marginally infected, moderately infected, or highly infected.

RESULTS

Pruning Weight

Significant differences in pruning weight among rootstocks were observed for each of the three growing seasons ($P < 0.05$). To interpret differences among rootstocks, Tukey's test was performed (Table 3.1). Vines grafted on GRN-2 had the highest pruning weight in 2014. Vines grafted on GRN-1, GRN-3, and GRN-4 had statistically similar pruning weights as vines grafted on GRN-2 during this growing season. Vines grafted on RS-9 had the lowest pruning weight in 2014 and were statistically similar to those grafted on 101-14, 3309C, Harmony, O39-16, RS-3, and St. George.

The rootstock O39-16 produced the highest pruning weight in 2018, and it was statistically similar to vines grafted on 1103P, 3309C, GRN-1, GRN-2, GRN-3, GRN-4, and

GRN-5. In 2018, vines grafted on RS-9 again produced the lowest pruning weight. Vines grafted on 101-14, Harmony, RS-3, and St. George were statistically similar to those on RS-9 in terms of pruning weight during this year.

GRN-4 produced the highest pruning weight in 2020 with a statistically similar performance as 1103P, 3309C, GRN-1, GRN-2, GRN-3, GRN-5, Harmony, O39-16, and RS-3. RS-9 maintained its consistent low vigor and again produced the lowest pruning weight for 2020. Its pruning weight for this growing season was statistically similar to that of vines grafted on 101-14, RS-3, and St. George.

Notably, GRN-1, GRN-2, GRN-3, and GRN-4 produced consistently high pruning weights over the three observed growing seasons, performing statistically similar to the rootstock with the highest pruning weight for any given growing season, or they were the rootstock with the highest pruning weight for that season. 101-14, RS-3, RS-9, and St. George produced consistently low pruning weights for the three observed growing seasons. O39-16 produced a low pruning weight in 2014 (performing statistically similar to the rootstock with the lowest pruning weight for that year) but produced high pruning weights in 2018 and 2020. The other four rootstocks (1103P, 3309C, GRN-5, and Harmony) ranged from intermediate to high during the three observed growing seasons.

Fruit Yield and Number of Clusters

Significant differences in fruit yield among rootstocks were observed for each of the three growing seasons ($P < 0.05$). To interpret differences among rootstocks, Tukey's test was performed (Table 3.2). Notable results regarding fruit yield include the consistent high yield from vines grafted on 3309C, GRN-1, and GRN-2. For all three observed growing

seasons, these three rootstocks performed statistically similar to the rootstock with the highest yield for that growing season, or they were the rootstock with the highest yield for that season. RS-9 produced the lowest yield for all three years, and RS-3 and St. George performed statistically similar to RS-9 for those years.

No significant differences in the number of clusters per vine for each rootstock were observed in 2014 ($P > 0.05$). However, significant differences were observed for the other two growing seasons ($P < 0.05$). Tukey's test was performed to interpret differences among rootstocks (Table 3.3). All rootstocks performed adequately during these two years, except for RS-3 and RS-9. During 2018 and 2020, vines grafted on these two rootstocks produced significantly fewer clusters than the vines that produced the most clusters. In 2018, vines grafted on St. George produced significantly fewer clusters than vines grafted on 3309C, GRN-1, GRN-2, GRN-3, GRN-5, and O39-16, but in 2020, vines grafted on St. George performed statistically similarly to all vines except RS-3 and RS-9.

Titratable Acidity

No significant differences in titratable acidity for each rootstock were observed in 2014 ($P > 0.05$). However, significant differences were observed for the other two growing seasons ($P < 0.05$). Tukey's test was performed to interpret differences among rootstocks (Table 3.4). Notable results include the consistent high titratable acidity in berries harvested from vines grafted on GRN-4 and O39-16. St. George had low titratable acidity in 2018 and 2020, having the lowest value in 2020 and performing statistically similar to the rootstock that produced the lowest titratable acidity in 2018 (Harmony).

Vine Infection Status

From the 265 vines tested in February 2021 (nearly ten years after the establishment of the trial), 110 vines tested positive, clearly indicating that GFLV was present at the site. Notably, vines grafted on St. George, a rootstock known to be highly susceptible to GFLV, had a positivity rate of 65 percent with 60 out of 93 vines tested to be positive (Table 3.5). Vines grafted on 1103P also had a positivity rate of 65 percent with 15 out of 23 vines testing positive. In contrast, vines grafted on O39-16 and GRN-1 had positivity rates of 4 percent and 8 percent, respectively (1 positive out of 25 vines, and 2 positives out of 25 vines, respectively). The other GRN rootstocks had positivity rates ranging from 42 percent (GRN-5) to 20 percent (GRN-2).

Vines that tested positive were classified as either marginally infected, moderately infected, or highly infected based on the Jenks natural breaks classification method (Table 3.5). For both St. George and 1103P, 35 percent of tested vines grafted on these rootstocks were classified as highly infected. Vines grafted on GRN-4 showed the next highest proportion of highly infected vines with 20 percent of vines classified as so. O39-16 had the lowest percentage of highly infected vines with only 4 percent of tested vines classified as highly infected (1 vine from 25 tested vines). This one vine is also the only vine grafted on O39-16 that tested positive. GRN-1 and GRN-2 had the next lowest proportions of highly infected vines with both at 8 percent.

ELISA results were also used to color-code a map of the rootstock trial according to infection status (Figure 3.2). The map indicates that there are certain “hot spots” in which the moderately infected and highly infected vines occur, particularly in rows 1 and 2. These hot spots are generally around where vines grafted on St. George and 1103P are located although

not all St. George and 1103P plots are highly infected. Notably, the GRN-3 plot in row 2 contains three vines that are highly infected and the other two vines in the plot are marginally infected.

DISCUSSION

Nematodes are a major problem in the grape-growing industry, particularly *Xiphinema index* for its vectoring of grapevine fanleaf virus, the causal agent of fanleaf degeneration. Once infested with *X. index* and GFLV, a vineyard will likely stay diseased due to the difficulty in eradicating the nematode population from the soil (Andret-Link et al., 2004). Nematicides tend to have limited effectiveness in deep soils, and many can contain toxic agrochemicals which prohibits their use in several countries (Abawi and Widmer, 2000). Additionally, *X. index* is very resilient and can retain GFLV over an extended period. Demangeat *et al.* (2005) were able to detect GFLV in *X. index* that was isolated from vineyard soil and stored for four years in the absence of host plants. The long-term survival of viruliferous *X. index* under such unfavorable conditions make control strategies such as fallow periods less effective, further emphasizing the need to develop resistant rootstocks.

Five rootstocks were released by UC Davis in 2008 that were selected for their ability to resist a wide range of nematodes (Ferris et al., 2012). All five rootstocks (named GRN-1 to GRN-5) resist *X. index*, but their ability to induce fanleaf tolerance (such as the case in O39-16) was unknown. In addition, because of their recent release, the overall performance of these rootstocks on a GFLV-infested site had not been thoroughly evaluated. The present study quantified the performance of the GRN rootstocks on a fanleaf site in the San Joaquin

Valley and compared their performance to several other rootstock standards, such as 1103P and O39-16.

The GRN rootstocks performed comparably to O39-16 in terms of vine performance during the three growing seasons evaluated for this study (Table 3.1, Table 3.2, and Table 3.3). Notably in 2018 and 2020, there was no statistical difference in pruning weight, fruit yield, and number of clusters per vine between each of the GRN rootstocks and O39-16 except for one case in which the fruit yield of vines grafted on GRN-1 in 2018 was statistically higher than that of vines grafted on O39-16. Given that O39-16 is the only commercially available rootstock that is known to perform well on fanleaf sites due to its ability to confer fanleaf degeneration tolerance to scions, these results are promising for the GRN rootstocks (Walker et al., 1994b).

GRN-1 especially shows promise due to its *Muscadinia rotundifolia* background. This species is the source of rootstock-induced tolerance observed in O39-16 (Walker and Jin, 1998). This finding was further validated in Chapter 2. Similar to the vines grafted on O39-16, vines grafted on GRN-1 in this study had a low GFLV positivity rate ten years after the establishment of the trial, especially compared to the positivity rate observed on vines grafted on St. George. Vines grafted on St. George (a rootstock known to be highly susceptible to GFLV) showed a positivity rate of 65 percent while vines grafted on GRN-1 showed a positivity rate of 8 percent. Although GRN-1 does not prevent infection altogether, the limited number of infected vines indicates that there may be benefits in the usage of GRN-1 on sites infested with *X. index* and GFLV. The slow uptake of the virus may delay infection significantly enough to reduce the overall impact of fanleaf degeneration over the lifespan of a vineyard grafted on this rootstock. There is also a possibility that GRN-1 can

also induce fanleaf tolerance to scions. The performance of vines grafted on GRN-1 should continue to be monitored on this site to determine its long term performance and potential to become an alternative for O39-16. These vines should also be closely monitored for fanleaf degeneration symptoms (such as poor fruit set), especially the one vine that tested positive in early 2021.

Although GRN-2 and GRN-3 do not contain *M. rotundifolia* in their background (both are hybrids with *V. rufotomentosa*, *V. champinii*, and *V. riparia* parentage), vines grafted on GRN-2 and GRN-3 also showed moderately low positivity rates (20 percent and 28 percent, respectively). Although not as low as vines grafted on O39-16 and GRN-1 (4 percent and 8 percent, respectively), these rates are not nearly as high as what was observed in St. George and 1103P (both at 65 percent). The moderately low infection rates of vines grafted on GRN-2 and GRN-3 may originate from the rootstocks' resistance to *X. index*. The nematode cannot successfully feed and reproduce on the rootstocks, thus there may be a lower number of nematodes in the plots with *X. index*-resistant rootstocks (Ferris et al., 2012). Future studies should sample for nematodes in each plot to better monitor nematodes. If nematode numbers are similar across all plots, there may be another mechanism causing these lower infection rates, such as GFLV resistance, which will be discussed below.

Like GRN-2 and GRN-3, GRN-4 is a hybrid with *V. rufotomentosa*, *V. champinii*, and *V. riparia* background. GRN-5 is a hybrid with *V. champinii* and *V. riparia* parentage. Although GRN-4 and GRN-5 performed comparably to O39-16 in terms of pruning weight, yield, and number of clusters, their positivity rate was noticeably higher than O39-16 and the other GRN rootstocks with GRN-4 having a 40 percent positivity rate and GRN-5 at 42 percent. These rootstocks' higher susceptibility to GFLV infection indicate that GRN-4 and

GRN-5 may have a lower resistance to GFLV compared to the other GRN rootstocks. Resistance is the plant's ability to suppress virus multiplication to a degree (either completely or partially) (Oliver & Fuchs, 2011). GFLV resistance was further described and explored in Chapter 1. Although the apparent lower level of GFLV resistance in these two rootstocks is concerning, the results in Chapter 2 indicate that there does not appear to be a relationship between GFLV resistance in a rootstock and that rootstock's ability to induce fanleaf tolerance to infected scions. The vines grafted on GRN-4 and GRN-5 should continue to be observed to determine if the rootstocks' higher susceptibility to GFLV infection impacts long-term vine growth and fruit production. Also, field data from infected vines and uninfected vines should be collected and analyzed separately to determine if there are differences between infected vines and uninfected vines. A lack of significant differences between the two groups would indicate the possibility of fanleaf degeneration tolerance imparted by the rootstock.

Due to their resistance to *X. index*, there is potential that O39-16 and the GRN rootstocks can act as natural nematicides and that when vineyards planted exclusively on these rootstocks are pulled after a generation, nematode populations may be very low or non-existent. There are many issues that may complicate this process, such as nematode life-span, the lateral movement of roots from adjacent vineyards on susceptible rootstocks, and the presence of cover-crop species that may support *X. index*. Further studies are needed on the use of *X. index*-resistant rootstocks as a method to eradicate *X. index* (and subsequently GFLV) from infested fields.

St. George is known to be susceptible to *X. index* and GFLV (Catalano et al., 1991, Van Zyl et al., 2012). As expected, vines grafted on this rootstock performed poorly in this

study with significantly lower pruning weights and yields than vines grafted on O39-16 in 2018 and 2020 (Table 3.1 and Table 3.2). This is most likely related to the high GFLV positivity rate of vines grafted on St. George (Table 3.5). Fanleaf degeneration is known to cause a progressive decline of infected vines, especially reducing yield (Andret-Link et al., 2004). The high susceptibility of St. George to *X. index* likely also caused the vines to weaken. *Xiphinema index* damages roots and prevents new root development which can severely stunt growth (Van Zyl et al., 2012).

1103P is also known to be susceptible to *X. index* (Goumas and Tzortzakakis, 1998, Gutiérrez-Gutiérrez et al., 2011). In this study, although 1103P showed similar GFLV infection rates as St. George, it did not perform as poorly (Table 3.5). Vines grafted on 1103P produced statistically similar pruning weights and fruit yields as O39-16 during the entirety of the study (Table 3.1 and Table 3.2). Although it is clear that *X. index* is present around these vines due to the high GFLV positivity rate of vines grafted on 1103P, the vines have not declined in the same manner as vines grafted on St. George. Due to vines grafted on St. George being planted at row ends and between each block, the nematodes may have preferred to feed on those roots rather than the roots of 1103P, though this remains to be investigated. GFLV infections on vines grafted on 1103P may have been caused by occasional feeding, but not significant enough feeding to cause extensive root damage. Future studies on this site should take notice of the vines grafted on St. George that are flanking the plots containing vines grafted on 1103P. If there is a distinct difference in vine growth and production between these two rootstocks in close proximity, there may be a possibility that there is preferential feeding by the nematodes. There is also a possibility that the feeding damage on vines grafted on 1103P is not yet severe enough to result in a decline

in vine health, but the vines will begin declining in the near future. This reduced and delayed feeding may have also deferred the onset of severe fanleaf degeneration symptoms, but further studies are needed.

101-14, 3309C, and Harmony are rootstocks that were included in this study due to their common usage in California. Notably, 3309C has poor resistance to *X. index*, and 101-14 and Harmony have moderate to high resistance to *X. index* (Bettiga et al., 2003). 3309C and Harmony performed statistically similar to O39-16 in terms of pruning weight and fruit yield in all cases except that Harmony produced a lower pruning weight than O39-16 in 2018 (Table 3.1 and Table 3.2). In contrast, 101-14 performed more poorly than O39-16 in most cases. It was beyond the scope of this study to test vines grafted on these rootstocks for GFLV, but further studies should do so to relate vine performance to infection status.

RS-3 and RS-9 are recently released rootstocks that exhibit resistance to dagger nematodes and root-knot nematodes (McKenry 2005a, McKenry 2005b). Both rootstocks performed very poorly in this study with extremely low pruning weights and fruit yields compared to all other rootstocks tested (Table 3.2). RS-9 is particularly weak, producing the lowest pruning weight and yield during all three growing seasons in this study. Although their infection status was not fully tested, their poor performance demonstrates that these rootstocks are unsuitable for this type of site and other rootstocks should be considered.

In addition to reducing fruit yield and shortening the lifespan of vines, GFLV also affects fruit quality by decreasing the titratable acidity of berries (Raski et al., 1983, Andret-Link et al., 2004). Although the titratable acidity values in this study are somewhat low for all the rootstocks (Table 3.4), this may be explained by the warmer climate in the Lodi wine region and the generally low titratable acidity of *V. vinifera* cv. Malbec (King et al., 2014).

Although some effect of rootstock was observed for titratable acidity, values from berries grown on GRN rootstocks were generally near the values for O39-16. Notably in 2020, berries harvested from vines grafted on St. George produced significantly lower titratable acidity values than those from all other rootstocks. This result is most likely explained by the high GFLV infection rates of vines grafted on St. George and the onset of fanleaf degeneration symptoms.

The highly infected vines observed in this study seem to be concentrated around specific areas of the trial, particularly plots with vines grafted on St. George and 1103P (Figure 3.2). This is expected since these two rootstocks are known to be susceptible to *X. index* (Bettiga et al., 2003). Not all plots containing St. George and 1103P are highly infested, which may also have to do with the fact that the nematodes are more concentrated on the side of the vineyard with rows 1 and 2. However, the existence of infected vines in the other three rows confirm that *X. index* is present in the entire trial. Notably, all three highly infected vines that are grafted on GRN-3 are located in the plot in row 2. The other two vines in that plot also tested positive but were classified as marginally infected. These five infected vines make up over 70 percent of the vines grafted on GRN-3 that tested positive. This may indicate that there are high populations of *X. index* in that particular plot, and GRN-3 succumbed more quickly to GFLV infection under this high nematode pressure. However, this disease incidence map is incomplete due to vines grafted on five of the thirteen rootstocks not being tested due to the study's primary focus on the GRN rootstocks. For a more complete overview of potential nematode and GFLV spatial distribution, all vines in the trial should be tested.

In addition to testing all the vines regularly to track the spread of GFLV on the site, additional fanleaf symptoms beyond reduced fruit yield (such as poor fruit set) should also be assessed on all infected vines to more definitively determine if any of the rootstocks can induce fanleaf tolerance to scions. This is especially important for infected vines grafted on GRN-1 due to the rootstock's *M. rotundifolia* background. The method described in Chapter 2 to quantify fruit set can be one method to assess fanleaf tolerance in addition to overall vine vigor and yield.

CONCLUSION

This study is the first to evaluate the performance of the GRN rootstocks on a site infested with *X. index* and GFLV. After ten years of growth, all five GRN rootstocks performed similarly to O39-16, the only commercially available fanleaf tolerant rootstock. The low GFLV infection rate in vines grafted on GRN-1 shows the potential for GRN-1 to become a viable alternative to O39-16. These results are encouraging, but additional studies to observe the long-term performance of these rootstocks are required to fully assess their suitability on fanleaf sites. Furthermore, these rootstocks should be tested on more sites with varying degrees of pest and disease pressure to gain greater confidence in the rootstocks' ability to act as a control measure against *X. index* and GFLV.

TABLES

Table 3.1. Pruning weight of *V. vinifera* cv. Malbec grown on thirteen rootstocks at Acampo, CA for three selected years. Pruning weight data for a given year were collected in February of the following year. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

	Pruning Weight (kg/vine)		
Rootstock	2014	2018	2020
101-14	0.855 de	1.141 cd	0.666 bc
1103P	1.022 bcd	1.343 abc	0.848 ab
3309C	0.922 cde	1.323 abc	0.858 ab
GRN-1	1.087 abc	1.327 abc	0.953 ab
GRN-2	1.239 a	1.563 ab	1.133 a
GRN-3	1.079 abc	1.303 abc	0.836 ab
GRN-4	1.151 ab	1.673 a	1.147 a
GRN-5	1.077 bcd	1.622 a	0.988 ab
Harmony	0.931 cde	1.191 bcd	0.896 ab
O39-16	0.935 cde	1.683 a	1.097 a
RS-3	0.809 de	1.052 cd	0.748 abc
RS-9	0.765 e	0.855 d	0.366 c
St. George	0.954 bcde	1.167 bcd	0.654 bc

Table 3.2. Fruit yields of *V. vinifera* cv. Malbec grown on thirteen rootstocks at Acampo, CA for three selected years. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

	Fruit Yield (kg/vine)		
Rootstock	2014	2018	2020
101-14	16.1 a	10.0 bc	12.5 cde
1103P	16.6 a	11.7 b	14.1 bcd
3309C	17.2 a	12.9 ab	16.8 ab
GRN-1	16.0 a	15.6 a	20.4 a
GRN-2	16.4 a	12.7 ab	17.8 ab
GRN-3	18.0 a	13.0 ab	15.1 bc
GRN-4	14.7 ab	11.7 b	15.4 bc
GRN-5	14.7 ab	11.8 b	14.5 bcd
Harmony	16.4 a	12.1 b	13.9 bcd
O39-16	16.3 a	12.4 b	17.3 ab
RS-3	14.5 ab	8.6 cd	9.5 ef
RS-9	11.2 b	6.7 d	7.5 f
St. George	15.5 ab	8.2 cd	10.9 def

Table 3.3. Mean number of clusters per vine of *V. vinifera* cv. Malbec grown on thirteen rootstocks at Acampo, CA for three selected years. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

	Clusters (mean no./vine)		
Rootstock	2014	2018	2020
101-14	99	83 abcd	95 ab
1103P	97	88 abc	99 a
3309C	98	93 a	109 a
GRN-1	87	98 a	109 a
GRN-2	102	93 a	106 a
GRN-3	105	92 ab	97 ab
GRN-4	88	87 abc	105 a
GRN-5	96	93 ab	98 a
Harmony	96	88 abc	101 a
O39-16	101	92 ab	104 a
RS-3	100	78 bcd	79 bc
RS-9	88	68 d	68 c
St. George	98	75 cd	92 ab

Table 3.4. Titratable acidity of *V. vinifera* cv. Malbec grown on thirteen rootstocks at Acampo, CA for three selected years. Letters indicate significant differences according to Tukey's test (alpha = 0.05).

	Titratable Acidity ($g \cdot L^{-1}$)		
Rootstock	2014	2018	2020
101-14	3.6	3.0 bcde	3.0 cd
1103P	3.6	3.0 bcd	3.1 bcd
3309C	3.5	3.1 bc	3.0 cd
GRN-1	3.7	2.7 defg	3.1 bcd
GRN-2	4.0	3.0 cdef	3.3 ab
GRN-3	3.4	3.5 a	3.2 bc
GRN-4	4.0	3.4 ab	3.3 ab
GRN-5	4.0	3.4 ab	3.2 bc
Harmony	3.7	2.6 g	2.9 d
O39-16	3.7	3.2 abc	3.5 a
RS-3	3.2	2.7 efg	3.0 cd
RS-9	3.7	3.2 abc	3.2 bc
St. George	2.7	2.7 fg	2.5 e

Table 3.5. ELISA results of *V. vinifera* cv. Malbec grown on GRN-1, GRN-2, GRN-3, GRN-4, GRN-5, 1103P, O39-16, and St. George at Acampo, CA after the 2020 growing season.

	Proportion of Vines			
Rootstock	Negative	Marginally Infected	Moderately Infected	Highly Infected
1103P	0.35	0.13	0.17	0.35
GRN-1	0.92	0	0	0.08
GRN-2	0.80	0.12	0	0.08
GRN-3	0.72	0.08	0.08	0.12
GRN-4	0.60	0	0.20	0.20
GRN-5	0.58	0.13	0.17	0.13
O39-16	0.96	0	0	0.04
St. George	0.35	0.13	0.16	0.35

FIGURES

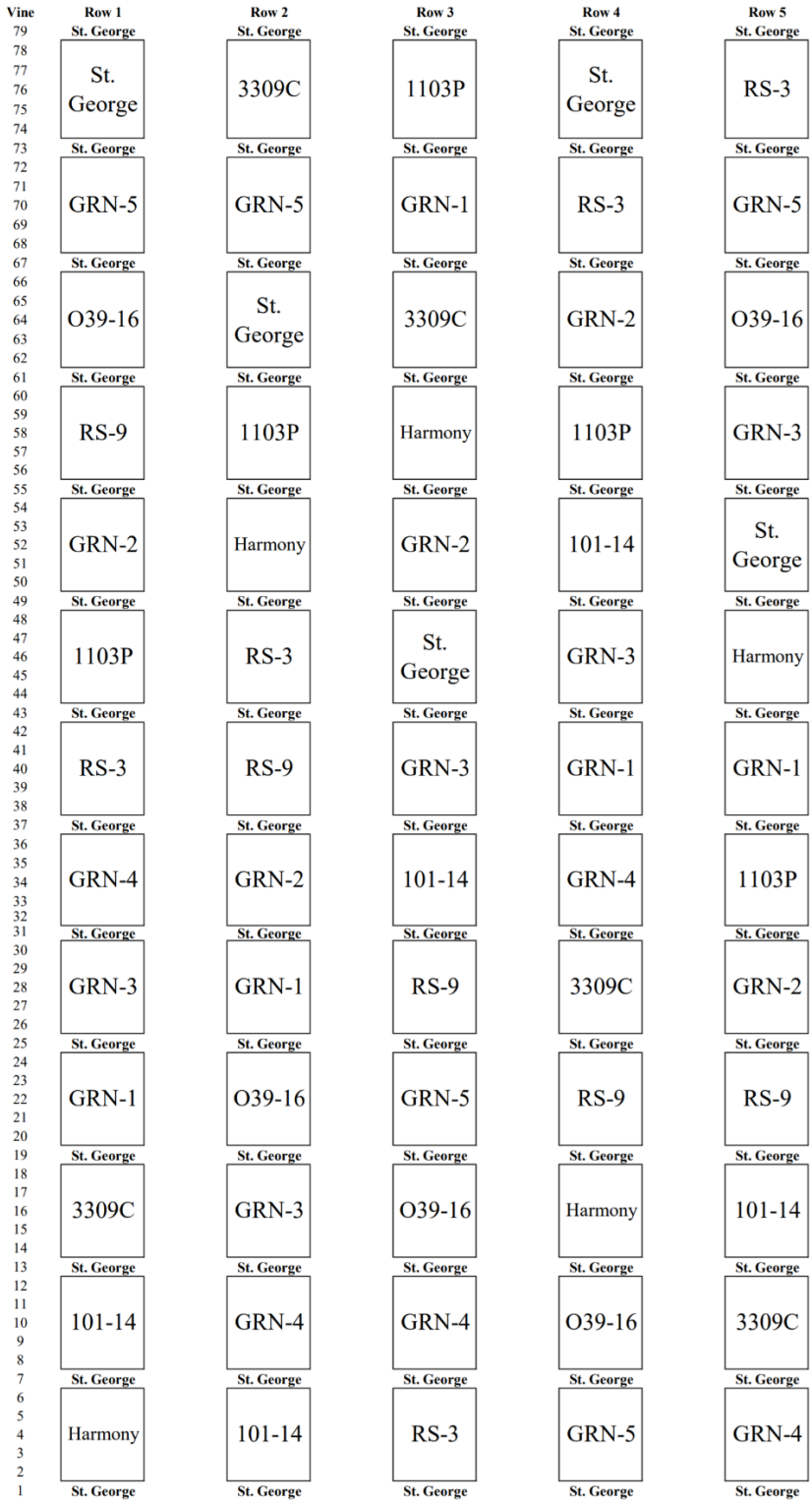
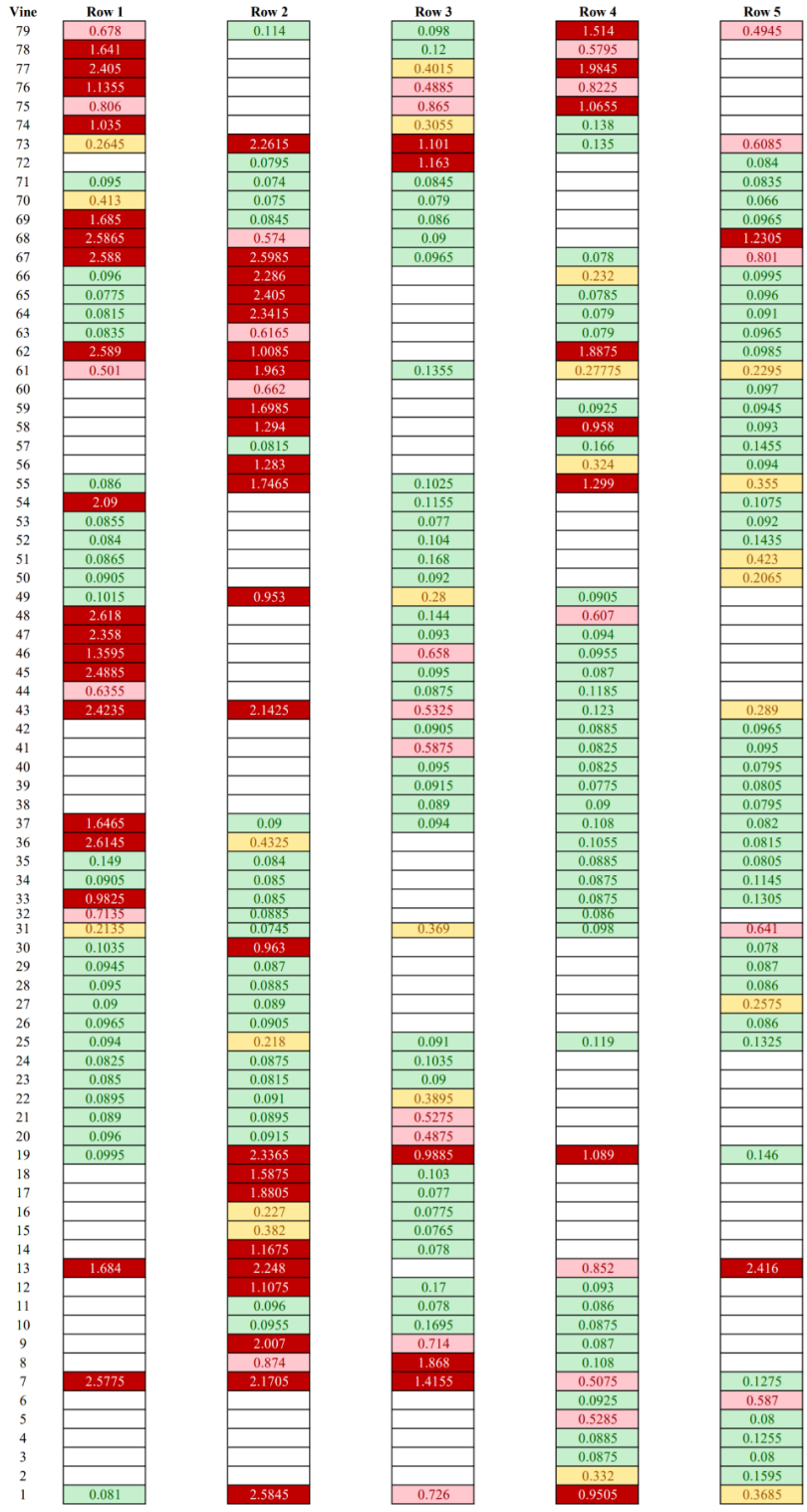


Figure 3.1. Experimental design of the rootstock trial. All vines are grafted with *V. vinifera* cv. Malbec and the indicated rootstock.



Vine	Row 1	Row 2	Row 3	Row 4	Row 5
79	0.678	0.114	0.098	1.514	0.4945
78	1.641		0.12	0.5795	
77	2.405		0.4015	1.9845	
76	1.1355		0.4885	0.8225	
75	0.806		0.865	1.0655	
74	1.035		0.3055	0.138	
73	0.2645	2.2615	1.101	0.135	0.6085
72		0.0795	1.163		0.084
71	0.095	0.074	0.0845		0.0835
70	0.413	0.075	0.079		0.066
69	1.685	0.0845	0.086		0.0965
68	2.5865	0.574	0.09		1.2305
67	2.588	2.5985	0.0965	0.078	0.801
66	0.096	2.286		0.232	0.0995
65	0.0775	2.405		0.0785	0.096
64	0.0815	2.3415		0.079	0.091
63	0.0835	0.6165		0.079	0.0965
62	2.589	1.0085		1.8875	0.0985
61	0.501	1.963	0.1355	0.27775	0.2295
60		0.662			0.097
59		1.6985		0.0925	0.0945
58		1.294		0.958	0.093
57		0.0815		0.166	0.1455
56		1.283		0.324	0.094
55	0.086	1.7465	0.1025	1.299	0.355
54	2.09		0.1155		0.1075
53	0.0855		0.077		0.092
52	0.084		0.104		0.1435
51	0.0865		0.168		0.423
50	0.0905		0.092		0.2065
49	0.1015	0.953	0.28	0.0905	
48	2.618		0.144	0.607	
47	2.358		0.093	0.094	
46	1.3595		0.658	0.0955	
45	2.4885		0.095	0.087	
44	0.6355		0.0875	0.1185	
43	2.4235	2.1425	0.5325	0.123	0.289
42			0.0905	0.0885	0.0965
41			0.5875	0.0825	0.095
40			0.095	0.0825	0.0795
39			0.0915	0.0775	0.0805
38			0.089	0.09	0.0795
37	1.6465	0.09	0.094	0.108	0.082
36	2.6145	0.4325		0.1055	0.0815
35	0.149	0.084		0.0885	0.0805
34	0.0905	0.085		0.0875	0.1145
33	0.9825	0.085		0.0875	0.1305
32	0.7135	0.0885		0.086	
31	0.2135	0.0745	0.369	0.098	0.641
30	0.1035	0.963			0.078
29	0.0945	0.087			0.087
28	0.095	0.0885			0.086
27	0.09	0.089			0.2575
26	0.0965	0.0905			0.086
25	0.094	0.218	0.091	0.119	0.1325
24	0.0825	0.0875	0.1035		
23	0.085	0.0815	0.09		
22	0.0895	0.091	0.3895		
21	0.089	0.0895	0.5275		
20	0.096	0.0915	0.4875		
19	0.0995	2.3365	0.9885	1.089	0.146
18		1.5875	0.103		
17		1.8805	0.077		
16		0.227	0.0775		
15		0.382	0.0765		
14		1.1675	0.078		
13	1.684	2.248		0.852	2.416
12		1.1075	0.17	0.093	
11		0.096	0.078	0.086	
10		0.0955	0.1695	0.0875	
9		2.007	0.714	0.087	
8		0.874	1.868	0.108	
7	2.5775	2.1705	1.4155	0.5075	0.1275
6				0.0925	0.587
5				0.5285	0.08
4				0.0885	0.1255
3				0.0875	0.08
2				0.332	0.1595
1	0.081	2.5845	0.726	0.9505	0.3685

Figure 3.2. ELISA results (OD_{405}) of vines grafted on GRN-1, GRN-2, GRN-3, GRN-4, GRN-5, 1103P, O39-16, and St. George after the 2020 growing season. Healthy vines are represented in green, marginally infected vines are represented in yellow, moderately infected vines are represented in light red, and heavily infected vines are represented in dark red.

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